



Call No. _____

Acc. No. 28070/36

SOIL SCIENCE

VOLUME XXIII

JANUARY - JUNE, 1927

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

CONTENTS

Sulfuric Acid Treatment of Cotton Seed. R. G. ARCHIBALD.....	1
Black Arm Disease of Cotton with Special Reference to the Existence of the Causal Organism <i>B. Malvacearum</i> within the Seed. R. G. ARCHIBALD.....	5
The Use of Collodion Sacks in Obtaining Clear Soil Extracts for the Determination of the Water-Soluble Constituents. W. H. PIERRE AND F. W. PARKER.....	13
The Occurrence of Yeasts in Soil. R. L. STARKEY AND A. T. HENRICI.....	33
Studies on Some Obligate Thermophilic Bacteria from Soil. WILLIAM A. FEIRER..	47
Minnesota Glacial Soil Studies: II. The Forest Floor on the Late Wisconsin Drift. FREDERICK J. ALWAY AND PAUL M. HARMER.....	57
Minnesota Glacial Soil Studies: III. Density of the Surface Foot in Forest and Prairie on the Late Wisconsin Drift. PAUL M. HARMER.....	73
Book Reviews.....	81
The Influence of Boron on the Growth of the Soybean Plant. GILBEART H. COLLINGS	83
Organic Matter Changes in Two Soil Zones, as Influenced by Difference in Form, Fineness, and Amount of Calcic and Magnesian Materials. W. H. MACINTIRE, W. M. SHAW AND ESTHER M. CRAWFORD.....	107
The Phenomena of Contraction and Expansion of Soils When Wetted with Water. GEORGE JOHN BOUYOUCOS.....	119
Colloidal Behavior of Soils and Soil Fertility: III. Cation Replacement and Saturation of Soil with Ca. J. S. JOFFE AND H. C. MCLEAN.....	127
A Study of the Factors Influencing the Efficiency of Different Forms of Nitrogen as Related to Soil Type and Cropping System in the Atlantic Coastal Plain Region: Part I. ARTHUR MATTHIAS SMITH.....	137
Fertility of a Soil as Related to the Forms of Its Iron and Manganese. P. H. BREWER AND R. H. CARR.....	165
Outgo of Calcium, Magnesium, Nitrates and Sulfates from High-Calcic and High-Magnesian Limes Incorporated in Two Soil Zones. W. H. MACINTIRE.....	175
Potassium Content of Plants as an Indicator of Available Supply in Soil. J. W. AMES AND R. W. GERDEL.....	199
Effects of Some Electrolytes on Kaolin and the Probable Relation to the Soil. JOHN R. SKEEN.....	225
Differences in the Heat of Reaction Between Artificial and Soil Gels of Silica, Alumina, and Iron with Hydroxides. GEORGE JOHN BOUYOUCOS.....	243
Book Review.....	249
A Research Professorship in Forest Soils at Cornell University.....	251
On the Existence of Daily Changes in the Bacterial Numbers in American Soil. H. G. THORNTON AND R. A. FISHER.....	253
Studies of the Removal of Nutrients from Subsoil by Alfalfa. C. E. MILLAR.....	261
The Microflora and the Productivity of Leached and Non-Leached Alkali Soil. J. E. GREAVES.....	271
The Normal Moisture Capacity of Soils. CHARLES F. SHAW.....	303
The Hydrometer as a New and Rapid Method for Determining the Colloidal Content of Soils. GEORGE JOHN BOUYOUCOS.....	319
Book Review.....	333
Effect of Fertilizers on the Germination of Seeds. JACOB L. MAXTON.....	335

The Hydrometer as a New Method for the Mechanical Analysis of Soils. GEORGE JOHN BOUYOUCOS.....	343
Effect of Moisture, Temperature, and Other Climatic Conditions on <i>R. Leguminosarum</i> in the Soil. S. C. VANDECAVEYE.....	355
Effect of Local Influences in Modifying the General Atmospheric Conditions. ALFRED SMITH.....	363
Method of Preparation of Soil Suspensions and Degree of Dispersion as Measured by the Wiegner-Gessner Apparatus. GEORG WIEGNER.....	377
Further Studies on Michigan Soils Profiles with Special Reference to Dispersed Materials. M. M. MCCOOL, A. G. WEIDEMANN, AND G. SCHLUBATIS.....	391
Factors Affecting the Hydrogen-Ion Concentration of Soils. L. D. BAVER.....	399
Book Reviews.....	415
Carbon Dioxide Evolution of Soil and Crop Growth. HENRIK LUNDEGÅRDH.....	417
The Seedling Plant Method of Determining Soil Nutrient Deficiency. J. W. AMES AND R. W. GERDEL.....	455
The Possible Rôle of Iron Depositing Bacteria in the Formation of Hard-Pan. C. S. MUDGE.....	467
A Study of the Soils of Hidalgo County, Texas, and the Stages of Their Soil Lime Accumulation. HERMAN W. HAWKER.....	475
A Study of the Colloidal Fraction of Certain Soils Having Restricted Drainage. W. L. POWERS.....	487
Book Reviews	493

ILLUSTRATIONS

PLATES

BLACK-ARM DISEASE OF COTTON WITH SPECIAL REFERENCE TO THE EXISTENCE OF THE CAUSAL ORGANISM *B. MALVACEARUM* WITHIN THE SEED

Plate 1. Fig. 1. Transverse section of Black Arm infected cotton seed showing bacilli in a resin canal	11
2. Section of Black Arm infected cotton seed with bacilli in the cotyledon tissues.	11

THE OCCURRENCE OF YEASTS IN SOIL

Plate 1. Twelve Species of Yeasts Found in Soils.	45
--	----

MINNESOTA GLACIAL SOIL STUDIES: II. THE FOREST FLOOR ON THE LATE WISCONSIN DRIFT

Plate 1. Illustrations Showing Character of Woods at Hamel and Taylors Falls ...	71
Fig. 1. Wood II of the Hamel group. The cordwood, shown in the foreground, had been cut between the time of sampling and that of taking the photograph, in September, 1919	71
2. Interior of Wood III at Taylors Falls, with dense growth of ferns in foreground.....	71
3. Interior of Wood III at Hamel	71

THE INFLUENCE OF BORON ON THE GROWTH OF THE SOYBEAN PLANT

Plate 1. The Type and the Extent of Boron Injury on the First Pair of Leaves of Representative Plants which Received Equivalent Quantities of Boron as Boric Acid, Potassium Borate, and Borax.	105
--	-----

POTASSIUM CONTENT OF PLANTS AS AN INDICATOR OF AVAILABLE SUPPLY IN SOIL

Plate 1. Effect of Ammonium Sulfate and Sodium Nitrate Additions on Growth of Wheat Seedlings.	217
Plate 2. Effect of Liming on the Growth of Wheat Seedlings.	219
Fig. 1. Unlimed Soil.....	219
2. Limed Soil.....	219
Plate 3. Growth of Wheat Seedlings on Soils from 5-year Rotation Plots.	221
Fig. 1. Pot 61, unfertilized soil; pot 65, plot 2, acid phosphate.....	221
2. Pot 69, plot 3, muriate potash; pot 73, plot 5, sodium nitrate.....	221
Plate 4. Growth of Buckwheat and Wheat in Several Soil Dilutions	223
Fig. 1. Buckwheat.....	223
2. Wheat.....	223

STUDIES OF THE REMOVAL OF NUTRIENTS FROM SUBSOIL BY ALFALFA

Plate 1. Fig. 1. These roots grew out around the bottom of the 8-inch cylinders and then turned upward and ramified in the surface soil	269
2. A large number of fibrous roots developed near the point of application of nutrient solution, at the 36-inch depth.....	269

THE HYDROMETER AS A NEW AND RAPID METHOD FOR DETERMINING THE COLLOIDAL CONTENT OF SOILS

Plate 1. Showing the Dispersing Machine, Cylinder and Hydrometer, in the Determination of Soil Colloids.....	331
--	-----

THE HYDROMETER AS A NEW METHOD FOR THE MECHANICAL ANALYSIS OF SOILS

Plate 1. Type of Hydrometer, Cylinder, and Mortar Employed.....	351
Plate 2. Curves Showing Rate of Settling of Soil Particles as Measured by Hydrometer	353

CARBON DIOXIDE EVOLUTION OF SOIL AND CROP GROWTH

Plate 1. Fig. 1. Arrangement for determination of the Soil Respiration.....	451
2. The experiment field in 1925.....	451
3. Interior of the field laboratory.....	451
Plate 2. Figs. 1 and 2. Sugar beets from the non-gassed glasshouse 1.....	453
3 and 4. Sugar beets from the gassed house 2.....	453

THE POSSIBLE RÔLE OF IRON DEPOSITING BACTERIA IN THE FORMATION OF HARD-PAN

Plate 1. Fig. 1. Four of the hard-pans used in the studies.....	471
2. Typical fermentation of the iron depositing bacteria studied.....	471
Plate 2. Fig. 1. Colonies obtained by plating powdered hard-pan on Harder's medium	473
2. Filaments within cavities of the hard-pan.....	473
3. Filament seen in figure 2 removed from the cavity and enlarged to 900X.....	473

A STUDY OF THE SOILS OF HIDALGO COUNTY, TEXAS, AND THE STAGES OF THEIR LIME ACCUMULATION

Plate 1. Fig. 1. The third stage of development.....	485
2. Caliche bed over 15 feet thick.....	485

TEXT FIGURES

MINNESOTA GLACIAL SOIL STUDIES: II. THE FOREST FLOOR ON THE LATE WISCONSIN DRIFT

Fig. 1. Map of Minnesota Showing Locations of Woods Sampled.....	58
2. Amount of Nitrogen per Acre in the Forest Floor and in the Underlying 6 Inches of Soil.....	66

MINNESOTA GLACIAL SOIL STUDIES: III. DENSITY OF THE SURFACE FOOT IN FOREST AND PRAIRIE ON THE LATE WISCONSIN DRIFT

Fig. 1. Densities of the Four Sections of the Twelve Fields.....	77
--	----

ORGANIC MATTER CHANGES IN TWO SOIL ZONES, AS INFLUENCED BY DIFFERENCE IN FORM, FINENESS, AND AMOUNT OF CALCIC AND MAGNESIC MATERIALS

Fig. 1. Organic-CO ₂ Changes Induced in a Loam Soil by Surface-Zone Incorporations of Ca(OH) ₂ , CaO-MgO, 80-100-Mesh Limestone and Dolomite at the Constant Rate of 2000 Pounds of CaO per 2,000,000 Pounds of Soil, Moisture-Free Basis, During 4 Years' Fallow Exposure without Stirring.....	108
--	-----

THE PHENOMENA OF CONTRACTION AND EXPANSION OF SOILS WHEN WETTED WITH WATER

Fig. 1. Dilatometer for Measuring Volume Contraction of Soils when Wetted with Water.....	120
---	-----

**A STUDY OF THE FACTORS INFLUENCING THE EFFICIENCY OF DIFFERENT FORMS OF NITROGEN
AS RELATED TO SOIL TYPE AND CROPPING SYSTEM IN THE ATLANTIC
COASTAL PLAIN REGION: PART I**

Fig. 1. Diagrammatic Cross-section of Cylinder and Case.....	153
--	-----

FERTILITY OF A SOIL AS RELATED TO THE FORMS OF ITS IRON AND MANGANESE

Fig. 1. Precipitation of Aluminum, Ferric and Ferrous Iron, Manganese and Magnesium as Hydroxides and Calcium Bicarbonate as Calcium Carbonate....	167
2. Nature of the Green Manganese Solution	170

OUTGO OF CALCIUM, MAGNESIUM, NITRATES AND SULFATES FROM HIGH-CALCIC AND HIGH-MAGNESIC LIMES INCORPORATED IN TWO SOIL ZONES

Fig. 1. Progressive Calcium Outgo, as Pounds of CaCO_3 -Equivalent per 2,000,000 Pounds of Soil, from Controls, Surface-Zone and Subsurface-Zone Incorporations of $\text{Ca}(\text{OH})_2$ and CaO-MgO during a 4-year Period.	179
2. Progressive Magnesium Outgo, as Pounds of CaCO_3 -Equivalent per 2,000,000 Pounds of Soil, from Controls, Surface-Zone Incorporations of $\text{Ca}(\text{OH})_2$ and CaO-MgO during a 4-year Period	179
3. Progressive Calcium-Magnesium Outgo, as Pounds of CaCO_3 -Equivalent per 2,000,000 Pounds of Soil, from Controls, Surface-Zone and Subsurface-Zone Incorporations of $\text{Ca}(\text{OH})_2$ and CaO-MgO during a 4-year Period	180
4. Four-year Outgo of Calcium, Magnesium, and Ca-Mg in Terms of CaCO_3 from Surface-Zone and Subsurface-Zone Incorporations of Hydrated Lime, Burnt Dolomite, and CaO-MgO Mixtures at the Rate of 3570 Pounds of CaCO_3 (2000 Pounds CaO) Per 2,000,000 Pounds of Soil	184

EFFECTS OF SOME ELECTROLYTES ON KAOLIN AND THE PROBABLE RELATION TO THE SOIL

Fig. 1. Final Volume of Sedimented Kaolin in Graduates at End of 24 Hours after Shaking with Molar Concentrations of Electrolytes Indicated	231
2. Resistance in Ohms of Solutions with and without Kaolin at Various Molar Concentrations	232
3. Resistance in Ohms of Solutions with and without Kaolin at Various Molar Concentrations	232
4. Rate of Migration of Kaolin Particles in $\pm\mu$ Per Second When Effected by Molar Concentrations of Electrolytes	233

ON THE EXISTENCE OF DAILY CHANGES IN THE BACTERIAL NUMBERS IN AMERICAN SOIL

Fig. 1. Correlation Between Simultaneous Samples Plated on Soil Extract Agar.	255
2. Correlation Between Simultaneous Samples Plated on Egg Albumen Agar....	256
3. Correlation Between Simultaneous Samples Plated on Mineral Salts Agar....	257
4. Daily Counts on Mineral Salts Medium	258

THE NORMAL MOISTURE CAPACITY OF SOILS

Fig. 1. Effect of Increased Amounts of Water. Sampled Five Days After Application	306
2. Effect of Time on Moisture Distribution When 2.5 Inches of Water Was Applied	308
3. Effect of Quantity on Distribution of Water, Sampled 54 Days after Application	310
4. Distribution of Water in Tubes Receiving a 6-inch Irrigation, after Prolonged Periods of Adjustment.....	312

EFFECT OF LOCAL INFLUENCES IN MODIFYING THE GENERAL ATMOSPHERIC CONDITIONS

Fig. 1. Mean Temperature Range Departures for 1913-1919.	367
2. Mean Maximum 1913-1919 Departure from Latitude Mean.....	369
3. August Maximum Temperatures.....	373
4. Mean August Temperatures.....	374

FACTORS AFFECTING THE HYDROGEN-ION CONCENTRATION OF SOILS

Fig. 1. Description and Reaction Profile of Wooster Silt Loam.....	400
2. Variations in Soil Reaction on Four Plots from April 27 to September 23.....	405
3. Effect of Surface Treatment on Subsurface Layers.....	411

CARBON DIOXIDE EVOLUTION OF SOIL AND CROP GROWTH

Fig. 1. Simple Apparatus for Analyzing the CO ₂ Concentration of the Air.. . . .	418
2. Apparatus for Estimating the Soil Respiration.....	418
3. Scheme of New Apparatus for Accurate Analysis of the CO ₂ Concentration in the Air.....	419
4. Tool for Drawing Air-Samples from the Soil.....	422
5. Borer for Drawing Soil Samples of a Definite Volume from Different Depths..	423
6. Soil Strata.....	425
7. Seasonal Fluctuations of the Soil Respiration, Carbon Dioxide Concentration among the Leaves, Carbon Dioxide Content in the Soil Air, and Climatic Factors—Oats, 1923.....	428
8. Seasonal Fluctuations of Soil Respiration, Carbon Dioxide Factor, and Climatic Conditions—Sugar Beets, 1925.....	429
9. The Effect of Large Quantities of Farmyard Manure on the Soil Respiration	431
10. The Carbohydrate Assimilation of Leaves of Sugar Beets in Relation to the Factors of Light, Carbon Dioxide, and Temperature.	433
11. System of Perforated Iron Tubes for Gassing of Field Plots	434
12. Arrangement for Gassing of Beets with Carbon Dioxide Evolved from Manure	436
13. Apparatus for Analyzing the Carbon Dioxide Content in the Field	438
14. Arrangement for Securing the Air Samples in the Field.	439
15. Diagram of the Diffusion Currents When the Carbon Dioxide Factor is Below the Standard Value.....	439
16. Diagram of the Diffusion Currents When the Carbon Dioxide Factor Exceeds the Standard Value.....	439
17. Diagram Showing the Parallelism Between Carbon Dioxide Factor and the Yield of Oats... ..	445
18. Diagram Showing the Parallelism Between Carbon Dioxide Factor and Yield of Sugar Beets.	445

THE SEEDLING PLANT METHOD OF DETERMINING SOIL NUTRIENT DEFICIENCY

Fig. 1. Potassium Assimilated by Different Crop Plants.	463
---	-----

A STUDY OF THE SOILS OF HIDALGO COUNTY, TEXAS, AND THE STAGES OF THEIR LIME ACCUMULATION

Fig. 1. Sketch Map of Hidalgo County, Texas, Showing the Location of Its Major Soil Areas.....	477
--	-----

A STUDY OF THE COLLOIDAL FRACTION OF CERTAIN SOILS HAVING RESTRICTED DRAINAGE

Fig. 1. Maximum Percolation on Dayton and Malheur Soils.....	488
2. Titration Curves of Soil Colloids.....	490

SULFURIC ACID TREATMENT OF COTTON SEED

R. G. ARCHIEALD¹

Wellcome Tropical Research Laboratories, Khartum

Received for publication January 22, 1926

In recent years, the sulfuric acid treatment of cotton seed has been advocated as a preventive measure for certain diseases attacking cotton, notably the bacterial disease known as Angular Leaf Spot, Black Arm, or Boll Rot, and the fungal disease known as Anthracnose.

The results obtained by the American workers Rolfs, Faulwetter, Ludwig and others justified an experimental trial being carried out on a small scale in the Sudan, more especially in relation to the control of Black Arm disease of cotton.

An initial series of experiments was carried out to note the effect of acid treatment on the germination of the seed. The seed used was Egyptian Sakel from the previous cotton crop, and had been ginned six months previously. The seed was soaked, for varying periods, in an enamel tin with different dilutions of sulfuric acid, then washed in water, dried, and sown two to three inches deep in moist soil contained in pots—20 seeds to each pot.

It is unnecessary to give the details of these experiments; suffice it to say that 500 gm. of cotton seed could be treated with 100 cc. concentrated sulfuric acid for 1 hour, washed in 2 liters of water for 10 minutes, either sown at once, or dried and then sown, with a result of 95 per cent germination. Seed in such a bulk was intimately brought in contact with the acid by mixing with a wooden spoon, and completely delinted in a short time. The final result showed slight charring of the seed coat to a black color, and in some instances, the seed was completely decapsulated without any apparent adverse effect on germination.

This experiment was modified by washing in water for longer periods, and it was found that 1 hour was the maximum period for washing without affecting germination. Longer periods of washing tended to swell the seed coat, and gave a lower percentage of germination.

Seed treated with concentrated sulfuric acid in the above proportions, washed for 10 minutes, dried, and stored for 6 months, showed, after sowing, 92 per cent of germination, whereas, seed similarly treated and washed for one hour in 2 liters of water, dried, and stored for six months gave 87 per cent germination.

¹ Director.

The acid treatment of seed whether concentrated or in dilutions of 1/20, 1/100, 1/200, 1/500 for periods longer than 1 hour affected adversely the germination of the seed. In periods of treatment less than, and up to, 1 hour, seedlings usually appeared above the soil in five days.

Comparative field observations were carried out, on a large scale, with seed treated in the proportion of 500 gm. of seed to 100 cc. concentrated sulfuric acid for fifteen minutes to half an hour, washed, dried, and sown two months later, and with seed untreated and sown under the same conditions. The treated seed showed better germination than the untreated, and resulted in a healthier plant with better vegetative growth. Data regarding the final yield of cotton are being collected.

SULFURIC ACID TREATMENT AS A PREVENTIVE MEASURE FOR BLACK ARM

It may be stated at the outset that sulfuric acid treatment of Black Arm infected cotton seed has not given the results claimed by American workers. Preliminary pot experiments were carried out; cotton seed from an infected source being treated with sulfuric acid and sown under conditions which excluded the possibility of infection from soil, water, or insects. Seed treated with concentrated sulfuric acid for 1 hour and 5 minutes yielded, in one experiment among others, 5 per cent of seedlings with typical angular leaf spot infection of the cotyledons.

Investigations by the writer have shown that the causal organism of Black Arm can be recovered and grown from the tissues internal to the seed coat; short of destroying the germination of the seed, it is difficult to conceive of acid treatment affecting the vitality of bacilli within the resin canals and tissues of the embryo. Organisms lying immediately below the seed coat could certainly be adversely affected by acid treatment. It would appear then, that at the most, a partial sterilization of the seed can result from sulfuric acid treatment.

Field observations by the writer show that there can be no doubt, however, that acid treatment exerts a beneficial effect, by delaying the manifestations of Black Arm disease and so by permitting the plant to obtain a good stand before infection is apparent. More information, however, is required regarding the predisposing factors concerned with the spread of the disease. Environment, climate, soil, and farming methods appear to play a major or minor part, and are being investigated.

SUMMARY

1. Experiments were carried out to test the effect of sulfuric acid treatment on the germination of cotton seed.

2. Seed treated in the proportion of 500 gm. to 100 cc. concentrated sulfuric acid, washed for 10 minutes in 2 liters of water, dried, and then sown yielded 95 per cent germination.

Seed treated in such a manner and stored for 6 months gave 92 per cent germination.

3. Seed treated with concentrated sulfuric acid for longer periods than one hour, or washed for longer periods than one hour after treatment was adversely affected as regards germination.

4. Field observations on treated and untreated seed showed better germination and plant growth in favour of the former.

5. Sulfuric acid treatment will not completely sterilize Black Arm infected seed but appears beneficial because it delays the manifestations of the disease in the cotton plant.

BLACK ARM DISEASE OF COTTON WITH SPECIAL REFERENCE TO THE EXISTENCE OF THE CAUSAL ORGANISM *B. MALVACEARUM* WITHIN THE SEED

R. G. ARCHIBALD¹

Wellcome Tropical Research Laboratories, Khartum

Received for publication January 22, 1926

In certain countries, where cotton is grown on a large scale, considerable losses in yield result from the ravages of a disease known as Angular Leaf Spot of Cotton, Black Arm, and Boll Rot, names which merely indicate different manifestations of the same disease and which may all occur at the same time in the same plant.

The causal organism *B. malvacearum* was first isolated by Smith (6), who proved its pathogenicity by infection experiments, and since then its causal relationship to the disease has been fully established by other workers.

As to the method of infection of the plant, conflicting views have been held by different observers. Hibbard (4) in 1910, and Jenkins (5) somewhat later, mention that infection may be carried in the seed, and Faulwetter (2) considers that seed may be a probable factor in primary infection of the plant but considers that wind-blown rain plays a more important part in disseminating the disease throughout a cotton crop. In America, the results of seed treatment by means of chemicals supported the view that the seed coat harbored the causal organism, but the records (1, 3) show that treatment, even with concentrated sulfuric acid, failed to sterilize completely infected seed.

As far as the writer is aware, no serious attempts have been made by investigators to prove whether infection was on the seed coat and its appendages, lint and fuzz, or whether the infection was within the seed, indeed, from the literature available, it appears to have been generally accepted that the infection was on the seed coat with its attached lint and fuzz.

As the question was of importance, especially from the point of view of seed treatment on a large scale, the writer, twelve months ago, carried out some investigations. Some preliminary experiments with cultures of *B. malvacearum* showed that the organism was endowed with feeble resisting qualities against sunlight, heat, and desiccation and all attempts to demonstrate the existence of spores in cultures proved negative.

With such facts, which confirmed the work of other observers, it was difficult to support the hypothesis that infection was maintained on the outer coat of

¹ Director.

the seed, and indeed, a large number of cultural experiments subsequently carried out with the lint, fuzz, and the outer coat of the seed, failed to show the presence of *B. malvacearum*.

A further experiment served to advance the hypothesis that infection was internal to the seed coat; seeds from infected plants were placed in a hot-air oven, and heated to a temperature of 70°C. for 1 hour, a temperature well beyond the thermal death point of the bacillus. The seeds were removed and placed in sterilized soil (which had previously been sterilized by heat at a temperature of 180°C.) in sterile pots, the soil watered daily with sterile distilled water, and the pots placed under conditions free from insect attack. Germination occurred and the young cotyledons soon showed a heavy infection with angular leaf spot, the bacillus being readily recovered from the lesions.

Attempts were then made to recover the bacillus from the tissues within the seed coat.

Several techniques were employed and abandoned, the difficulty being in sterilizing completely the outer coat of the seed without affecting the interior. The use of alcohol, however, surmounted this difficulty.

Ginned cotton seeds, i.e. seeds which had been collected from cotton plants attacked with black arm twelve months previously, were placed in absolute alcohol for 3 minutes, removed with a pair of sterile forceps, and ignited in a flame in order to sterilize the outer coat of the seed. When the alcohol was completely burnt off, the seeds were then dropped into tubes of sterile distilled water and incubated at a temperature of 31 to 33°C. for 48 hours; by this time the seed coat had considerably softened, and in many instances had split. A sterile glass rod was then inserted into the tubes containing the seeds and distilled water, and the seeds were thoroughly crushed up. The tubes were then incubated for 24 to 36 hours at 31°C. and subcultures prepared on agar slopes.

In some instances, a pure culture of *B. malvacearum* was obtained in 20 per cent of seed taken from infected plants, the presence of *B. malvacearum* obtained from seed by this method was always verified by a study of the cultural and other characters of the organism, by its biochemical reactions, and finally by inoculation experiments on cotton seedlings in order to test its pathogenicity.

Having proved that infection was within the seed coat, attempts were made to section the seed to demonstrate the site of the organism contained therein.

Preliminary treatment of the seed with nitric acid was necessary to soften the coat, followed by fixation in graded alcohols, by clearing, and by embedding in paraffin. Sections were made and stained by Gram's method, thionin blue, and haematoxylin. Examination showed the organisms occupying the resin canals (plate 1, fig. 1) and the cotyledonous tissue (plate 1, fig. 2).

The same technique was employed in recovering the causal bacillus from the plant tissues below the site of lesions. Cotyledon leaves showing typical

angular leaf spot were removed and their supporting stems examined with a lens for evidence of gross lesions. Stems, free from lesions, were soaked in absolute alcohol, ignited, and then crushed in sterile distilled water, and agar cultures prepared. By this somewhat rigorous technique, it was possible to obtain *B. malvacearum* in the tissues and sap of apparently healthy areas below infected lesions; this was applied with similar results to tissues of the main stems supporting infected bolls.

It would appear then from these laboratory experiments, that infected seed was capable of producing infected plants and that the bacillus could be isolated from apparently uninfected tissues below black arm lesions, indicating possible latency on the part of the organism—a point which appears to be supported also by field observations.

The other likely sources of infection to the plant may be considered under the following headings: (a) soil, (b) water, (c) insects, and (d) ginning factories. As regards soil, the writer has carried out a large number of examinations of samples, taken at various depths, of cultivated and fallow soil, and has, so far, failed to recover *B. malvacearum*; the same remarks hold good for water samples taken from irrigation canals, and collections of standing water in cotton areas attacked with black arm. The feeble powers of resistance of the organism to such adverse conditions as sunlight, desiccation, and high temperature, as hold in the Sudan, make it difficult to accept any hypothesis indicating the soil as a reservoir of the virus. As regards the disease being insect borne, no epidemiological evidence exists in the Sudan to support such a view. In the early stages of the cotton plant, the flea beetle is the predominant pest, its ravages are obvious, yet plants free from attack show evidence of disease, and plants attacked show no signs. The same remarks apply equally to such cotton pests as thrips, aphids, stemborer, and white ants. Moreover, laboratory examinations of these insects have so far failed to incriminate any of them as vectors of the causal organism of black arm. The possibility of infection being maintained in ginning factories has been considered, a hypothesis which may be dismissed in view of the delicate nature of the organism concerned.

Faulwetter's theory that the disease is disseminated by the combined action of wind and rain is not supported by the extensive and careful field observations carried out by Bedford.²

No other plant hosts have been found either manifesting the disease, or harboring the bacillus; indeed, the writer has failed so far to reproduce lesions by culture inoculation into plants other than cotton. It would appear, therefore, that *B. malvacearum* is specific for the cotton plant.

The factors predisposing to the manifestation of the disease—soil, climate, and imperfect agricultural methods—are at present being investigated. The problem is beset with difficulties in view of what has been stated regarding

² Gezira Entomologist.

the possible latency of manifestations of infection. In this connection, investigations are now proceeding to ascertain whether healthy plants harbor the virus without showing evidence of infection. Field observations have shown that certain varieties of American rain grown cotton are more resistant to attack than others, a resistance shared also by rogue cotton and some ratoon cotton. It may be that such resistance is dependent on the pH reaction of the sap, a hypothesis worthy of investigation.

Seed treatment, by means of sulfuric acid, has been advocated as a remedial measure for the disease but, in the writer's experience, has not proved the success claimed by American workers. In view of the writer's investigations regarding infection within the seed coat, it is difficult to conceive any form of seed treatment, short of destroying the germination power of the seed, effectually sterilizing infected seed. Sulfuric acid treatment appears beneficial to the germinating properties of the seed, and assists by producing a healthier plant in its early stages, but experience has shown that such a plant, in its later stages of growth, often shows manifestations of the disease. The writer is of the opinion that more information regarding the predisposing factors of the disease is required, and when this knowledge is acquired, it will be possible to grow and maintain a healthy type of plant capable of resisting, or throwing off, infection when once attacked.

SUMMARY

1. Investigations have shown that, in Black Arm disease of cotton, the causal organism can be recovered from the tissues within the seed coat.
2. A technique is described for recovering the bacillus from the seed tissues.
3. The feeble resisting powers of the organism toward such adverse conditions as strong sunlight, desiccation, and high temperature render it unlikely that the outer coat of the seed, with its lint and fuzz, harbors infection.
4. The bacillus can be recovered from apparently healthy tissues below black arm lesions.
5. The seed appears to be the main source of infection.
6. The causal organism has not been found in soil or water, and the epidemiology of the disease does not favor the hypothesis that the disease is insect borne.
7. No hosts other than the cotton plant have been found.
8. Seed sterilization by means of concentrated sulfuric acid has yielded disappointing results. A more effectual way of attacking the problem is to ascertain the factors that predispose to manifestations of infection, and to raise a healthy type of plant capable of resisting as well as of throwing off infection when attacked.

REFERENCES

- (1) BROWN, J., AND GIBSON, F. 1925 A machine for treating cotton seed with sulphuric acid. *Ariz. Agr. Exp. Sta. Bul.* 105.

- (2) FAULWETTER, R. C. 1917 Wind blown rain, a factor in disease dissemination. *Jour. Agr. Res.* 10: 639-648.
- (3) FAULWETTER, R. C. 1919 The angular leaf spot of cotton. S. C. Agr. Exp. Sta. Bul. 198.
- (4) HIBBARD, R. P. 1910. Cotton disease in Mississippi. Miss. Agr. Exp. Sta. Bul. 140.
- (5) JENKINS, F. W. 1919 Cotton and some of its diseases and insects. Fla. Agr. Exp. Sta. Ext. Bul. 15.
- (6) SMITH, ERWIN 1920 The Angular Leaf Spot of Cotton. Bacterial Diseases of Plants. Philadelphia and London.

PLATE 1

FIG. 1. Transverse section of Black Arm infected cotton seed showing bacilli in a resin canal. $\times 960$.

FIG. 2. Section of Black Arm infected cotton seed with bacilli in the cotyledon tissues. $\times 800$.

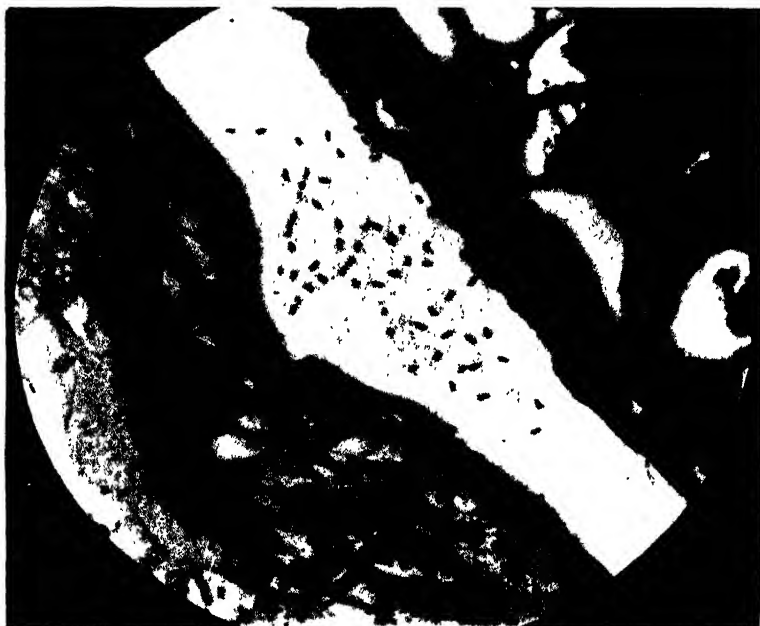


FIG. 1

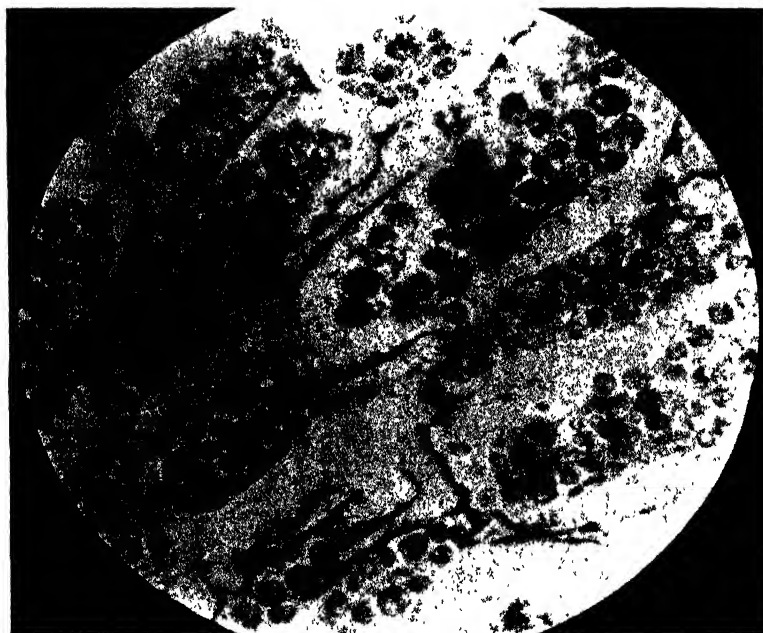


FIG. 2

THE USE OF COLLODION SACKS IN OBTAINING CLEAR SOIL EXTRACTS FOR THE DETERMINATION OF THE WATER-SOLUBLE CONSTITUENTS¹

W. H. PIERRE AND F. W. PARKER

Alabama Agricultural Experiment Station

Received for publication April 12, 1926

INTRODUCTION

A clear extract, essential in the analysis of the water-soluble constituents of the soil, is especially important where colorimetric methods are to be used. Difficulties in obtaining soil extracts unaltered in composition and free of all colloidal material, emphasize the desirability of a simple method that could be used in obtaining clear extracts for the determination of the water-soluble constituents of soils.

The methods used in obtaining soil extracts are: centrifuging or ordinary filtering with or without the use of flocculating agents, filtration by suction, filtering through Pasteur-Chamberland filters, and dialysis through collodion sacks.

It is generally recognized that ordinary filtering and centrifuging of soil suspensions do not give clear extracts with many soils. In order to overcome this difficulty various agents have been used to flocculate the colloidal particles that usually pass through the filter paper or remain in suspension. Although flocculents have proved very successful, they cannot be used in obtaining soil extracts for the colorimetric determination of H-ion concentration, nor for a determination of the basic constituents of the soil. In the latter case there is always the possibility of solubility effects as a result of base exchange.

The suction filtering method is based on the principle of untrafiltration through a thin layer of soil deposited on the filter paper, and, as recently used by one of the authors (21), gives a clear extract very readily. This method, although simple, requires a little care in manipulation, and is rather time consuming where a large number of extracts is to be obtained.

The Pasteur-Chamberland filter method gives large amounts of clear extract, but requires rather expensive equipment. Moreover, it cannot be used in H-ion work nor, as was recently shown by Parker (19), where phosphorus is to be determined in the extract. In order to study further the influence of the Pasteur-Chamberland filter on the composition of the extract the following

¹ Published with the permission of the Director of the Alabama Agricultural Experiment Station.

experiment, the results of which are given in table 1, was conducted. Extracts were prepared by dialysis from two soils which gave extracts of widely different composition with respect to several ions. The extracts were passed through thoroughly washed filters and the change in composition was determined. The filters were then washed with 300 cc. of distilled water, and a portion of the original extract from the other soil was passed through the filters. For example, a non-acid extract rather high in phosphorus was filtered through filters 5 and 6. Then these two filters were washed and used with an acid soil extract, low in phosphorus. In this manner the influence of filters used with one extract upon another extract subsequently filtered was determined.

TABLE 1
The composition of the soil extracts as influenced by Pasteur-Chamberland filters

SOIL NUMBER	SOIL	TREATMENT OF DIALYZED EXTRACT	SPECIFIC RESISTANCE	H-ION CONCENTRATION	PHOSPHORUS	CALCIUM	POTASSIUM	NITRATE NITROGEN
			<i>ohms</i>	<i>pH</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
27	Silt loam	None	10,475	6.60	1.55	11.6	3.4	3.7
		Passed through filters 5 and 6*	11,420	6.65	1.25	11.1	5.4	3.7
		Passed through filters 7 and 8†	11,700	6.62	1.25	9.7	5.5	3.7
28	Silt loam	None	14,810	4.70	0.05	4.7	6.6	3.1
		Passed through filters 7 and 8*	14,615	5.73	0.04	5.6	6.4	3.1
		Passed through filters 5 and 6†	14,810	5.80	0.12	5.5	7.5	3.1

* Filters were washed with 800 cc. distilled water before filtering the extract.

† Filters were those used under * for the other soil but were first washed by passing 300 cc. of distilled water through them.

It will be seen from table 1 that the filters had a marked effect on the phosphorus and H-ion concentration of the extracts. Thus, the H-ion concentration of soil 28 was changed from pH 4.7 to pH 5.7 by filtering. The phosphorus content of the extract was little affected with one filter whereas with the filter that had previously been used with a soil high in phosphorus, the phosphorus content of the extract was more than doubled as a result of passing through the filter. In soil 27, the filters absorbed about 32 per cent of the phosphorus in the extract, whereas the H-ion concentration was not affected. The calcium and potassium contents of the extract were affected to a less extent, although the data indicate that the filters may either retain or give up appreciable amounts of calcium and potassium. The nitrate content, as would be expected, is not affected by filtering.

The collodion sack method of obtaining extracts has been used in biological studies for a long time, but it has not been used extensively in soil investiga-

tions. Knight (11), Mirasol (16), Denison (5), and Magistad (15) used collodion sacks in a study of the nature of the aluminum compounds in the soil extract. As far as the authors know, Parker and Tidmore (20), working in this laboratory, were the first to use the method for obtaining soil extracts. They made extensive use of it in their studies on the phosphorus content of the soil solution and soil extracts. Their method, in brief, consisted in placing the collodion sack containing the soil suspension in a covered beaker containing a definite amount of water for eighteen to twenty hours to allow equilibrium to become established between the inside and outside solutions. The contents of the sack and beaker were shaken occasionally.

Because of the apparent possibilities of this method in overcoming some of the objections of the common methods of obtaining soil extracts, this study was undertaken. This paper presents the results of a study made with regard to:

(a) The method of preparing sacks and extracts, (b) the time necessary for the establishment of equilibrium between the solution inside and outside of the sacks, and (c) the use of the method in determinations of the H-ion concentration, nitrate nitrogen, phosphorus, and calcium content of soil extracts.

METHODS

The collodion sack method of obtaining a soil extract is based on the principle of dialysis or diffusion of soluble material through a collodion membrane. The soil suspension is placed in a collodion sack, which is then placed in water, and equilibrium is allowed to become established between the inside and outside solutions. After equilibrium is established, the inside and outside solutions are apparently of the same concentration with respect to all ions determined in soil extracts. Apparently there is no Donnan equilibrium established to cause a difference in concentration of any ion in the inside and outside solutions. This may be explained by assuming that there are no non-diffusible ions in the soil suspension. Subsequent data will show that the concentration of all ions studied was the same in the inside and outside solutions after equilibrium was established.

Preparation of sacks

In the preparation of the collodion sacks Loeb's (14, p. 109) procedure was used with some minor modifications. The sacks were made in flasks, the size depending on the size of sack desired or the amount of suspension to be dialyzed. Thus, where only about 50 cc. of soil extract was desired, as for the determination of nitrates and of H-ion concentration by colorimetric methods, 150-cc. extraction flasks were found very satisfactory. These flasks give sacks with wide necks through which the soil can be readily poured. When more extract was needed, sacks made in 500-cc. Erlenmeyer flasks were used. The procedure for making the sacks is as follows:

Merck's Blue Label collodion is poured into a thoroughly cleaned flask, which has been rinsed with 95 per cent alcohol to remove all water. It is essential that the collodion solu-

tion be kept free of water, for sacks made from a solution containing even small amounts of water are opaque and weak. While rotating the flask slowly by hand the collodion is poured back into the bottle during a period of 2 minutes. The flask now has a thin, uniform layer of collodion on the inside, which is allowed to dry by placing the flask in an inverted position in a ring stand for 8 to 10 minutes. It is then placed under a faucet and washed by allowing water to run into it 3 to 5 minutes. The water is then poured out, the edges of the neck of the collodion membrane are loosened a little from the neck of the flask, and water from the tap is allowed to run between the sack and flask. This procedure frees the sack from the walls of the flask, and the sack can then be readily removed and placed into a jar of water until used. By adding a little toluene to the water, mold growth is prevented and the sacks may be kept practically indefinitely.

Where a number of sacks are to be made, the authors work with six flasks. While the membrane is being formed in one flask, three flasks are drying and two are being washed. Using this system one person can make 15 to 20 sacks an hour.

The viscosity of the unused collodion gradually increases because of the evaporation of the solvent. As a result the sacks made from this solution become too thick. When this condition is noticed, ether must be added to the solution. The amount of ether needed can be readily estimated after a little experience. By the addition of ether as it becomes necessary, 75 to 90 sacks (made in a 150-cc. extraction flask) can be made from one pound of collodion.

Methods of controlling the permeability of the collodion membranes have been the subject of several investigations. It was first pointed out by Bigelow and Gernerling (1) that the longer collodion membranes are allowed to dry, the less permeable they become. Brown (2) found that if dry sacks are placed in different strength solutions of alcohol, those in the more concentrated alcohol solutions become more permeable and fragile than sacks placed in weaker solutions of alcohol. Schoep (22) found that sacks are more permeable if a little oil or glycerine is added to the collodion from which the sacks are made. Among others who worked on the permeability of collodion membranes are: Nelson and Morgan (17), Browne, R. J. (3), Eggerth (6), and Farmer (8).

Most of these workers, however, were working with membranes to be used for ultrafiltration. For dialysis work, as used ordinarily, it seems that the simple procedure used by Loeb in all his work and outlined above gives sacks of good permeability along with a fair degree of strength. In some preliminary work the writers found that the permeability of the sacks was practically the same whether they were dried two or thirty minutes before washing.

Procedure used for obtaining the soil extracts or dialysate

In the preparation of the soil extracts the sacks were removed from the jar, rinsed in distilled water and placed in the same kind and size of flasks as those in which they were made. The soil was then poured into the sacks by means of a short, wide-stem "powder-funnel" and the required amount of water added partly to the inside and partly to the outside of the sack. Where the extracts were desired for the determination of nitrates and of H-ion concentration only, 1:2 extracts were used, one-half of the water being added to the inside of the sacks. For the determination of all other ions where large amounts of extract were desired, 1:5 soil-water extracts were obtained. In that case only one-fourth of the water was added to the inside, the remaining water being added to the outside of the

sack. Thus large amounts of extracts were obtained. The necks of the sacks were then folded against the necks of the flasks, clean rubber stoppers inserted, and the flasks shaken. The shaking was done by hand and was repeated every hour during the day. After a period of eighteen hours the dialysates were removed from the sacks and used for the various analyses.

The use of flasks closed with rubber stoppers instead of beakers is not only highly desirable if the H-ion determination of the dialysates is to be determined, but it facilitates shaking and also makes possible the using of a mechanical shaker for the continuous shaking of the system. As subsequent data will show, the shaking of the flasks by means of a drawer type mechanical shaker causes the establishment of an equilibrium between soil and water in a shorter period of time. If hand shaking is to be used, however, covered beakers can be used as well as flasks. With the use of beakers it was found convenient to place water on the inside and the soil suspension on the outside of the sacks.

The sacks are easily cleaned and can be kept in a jar of water until again used. It was found that a sack may be used six to ten times, provided it is not allowed to dry.

Time required for the establishment of equilibrium

The time required for the suspension and dialysate to come to equilibrium depends largely on two factors; namely, the kind of ion being considered, and the kind and amount of shaking to which the system is subjected. Different ions have different diffusion velocities and they also come to equilibrium with the soil at different rates, because of variations in solubility. For the purpose of this study the ions are considered in two groups; first, readily soluble ions such as hydrogen, chloride, and nitrate; second, ions with which there may be considerable solubility effects, such as phosphate, calcium, and potassium. Shaking materially influences the time required for the establishment of an equilibrium and is one of the most important factors to consider in a study of the method. As a result of preliminary work it was apparent that somewhat different procedures should be used in the equilibrium studies for the two classes of ions; therefore, the two classes of ions will be considered separately.

Easily soluble constituents. As a preliminary study, three methods of shaking—hand shaking and two types of mechanical shaking—were compared with regard to their effect on the rate of establishment of equilibrium. In the hand method of shaking, the flasks were shaken for 1 minute at 15 minute intervals. Shaking by means of the Camp rotary shaker and the drawer shaker was continuous. With both the mechanical shakers the gearing was reduced to give one complete cycle per second. The nitrate content of the dialysates was determined by the phenoldisulfonic acid method, and the H-ion concentration by the colorimetric method.

It will be seen from table 2 that drawer shaking causes a more rapid diffusion of ions into the dialysate than does either of the other two methods. Thus the average nitrate content in the dialysates after one hour is 25.3 p.p.m. with

hand shaking and 31.5 p.p.m. with drawer shaking. Similarly, in a comparison of the H-ion concentration and of the specific resistance of the dialysates, the drawer type shaking causes the most rapid diffusion of ions, whereas the rotary shaking usually gives values intermediate between the two other methods. The data in this table, however, as will be shown later, do not always represent the final equilibrium of hydrogen and nitrate ions.

As a result of this preliminary study it seemed desirable to determine the time necessary for the establishment of equilibrium with the hand shaking and with the drawer shaking methods. Since rotary shaking was inferior to drawer shaking it was not used in subsequent experiments. The equilibrium of nitrate ions and of hydrogen ions between the dialysate and the suspension

TABLE 2

*H-ion concentration, specific resistance and concentration of nitrate nitrogen in dialysate after shaking the flasks for one hour by different methods.**

SOIL NUMBER	SOIL	H-ION CONCENTRATION			NITRATE NITROGEN			SPECIFIC RESISTANCE		
		Method of shaking			Method of shaking			Method of shaking		
		Hand	Rotary	Drawer	Hand	Rotary	Drawer	Hand	Rotary	Drawer
		pH	pH	pH	p.p.m	p.p.m	p.p.m.	ohms	ohms	ohms
1	Norfolk sandy loam	5 23	4 85	4 90	67 9	72 2	72.5	6,480	6,920	6,050
2	Sumter clay	7.23	7 58	7.63	3.8	4 1	4 6	18,010	14,430	14,235
3	Gray silt loam	4 58	4 58	4 58	7 1	6 9	8.4	25,950	26,315	21,680
4	Greenville sandy loam	5 03	5 05	5 03	26 6	36 1	41 8	17,065	15,275	12,295
5	Dekalb fine sandy loam	6 08	6 20	6 18	16 0	15 9	22 6	20,550	18,410	14,215
6	Brown silt loam	5.88	5 88	5 85	30 2	34 3	39.2	14,130	10,675	9,840
Average of all soils					25 3	28 3	31.5	17,031	15,375	13,053

* Nitrate nitrogen calculated on basis of dry soil.

inside of the collodion sack was determined after 2 and 12 hours of drawer shaking and after 4, 18, and 24 hours of hand shaking of the system. As in the previous experiment, the drawer shaking was continuous, whereas the hand shaking was at intervals of 15 minutes for the first hour and of 1 hour thereafter, except during the night (between the fourth and eighteenth hours). The soil-water ratio used was 1:2, one-half the water being added to the inside of the sack. After the various periods of time, the dialysate was removed for analysis. The soil suspension from inside the sack was filtered by means of a modified suction apparatus, consisting of a Shaw (24) filter flask and a Hirsch funnel, as devised by Truog (26). Nitrate and hydrogen ions were determined in the filtered extract and in the dialysate. The data as given in table 3 represent the average of duplicate determinations.

TABLE 3

*Nitrate nitrogen content and H-ion concentration of soil extracts after hand and drawer shaking of flasks for different periods of time**

SOIL NUMBER	SOIL	METHOD OF SHAKING	TIME OF SHAKING	NITRATE NITROGEN		H-ION CONCENTRATION	
				In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack
			hours	p.p.m.	p.p.m.	pH	pH
1	Norfolk sandy loam	Hand	4	67.3	74.6	5.00	4.90
			18	70.8	71.1	4.95	5.00
			24	69.4	70.5	4.95	4.85
		Drawer	2	65.7	69.1	5.00	5.00
			12	72.9	72.4	4.90	4.90
2	Sumter clay	Hand	4	5.6	5.9	7.65	7.60
			18	5.8	5.3	7.65	7.65
			24	5.3	5.2	7.65	7.70
		Drawer	2	5.1	5.6	7.60	7.65
			12	6.1	5.9	7.70	7.70
3	Gray silt loam	Hand	4	7.2	8.3	4.60	4.60
			18	8.5	8.5	4.60	4.60
			24	7.8	8.6	4.60	4.60
		Drawer	2	8.7	8.8	4.60	4.60
			12	8.5	8.2	4.55	4.60
4	Greenville sandy loam	Hand	4	45.2	49.9	5.05	5.10
			18	50.1	50.3	5.05	5.05
			24	48.3	51.1	5.10	5.10
		Drawer	2	47.0	47.3	5.05	5.05
			12	49.3	49.3	5.05	5.00
5	Dekalb fine sandy loam	Hand	4	21.4	22.6	6.45	6.45
			18	20.8	22.8	6.50	6.40
			24	22.6	21.9	6.50	6.45
		Drawer	2	23.8	23.8	6.45	6.50
			12	25.3	21.9	6.50	6.50
6	Brown silt loam	Hand	4	52.4	61.4	6.00	6.00
			18	60.4	57.2	6.05	6.00
			24	58.6	58.8	6.05	6.05
		Drawer	2	58.0	58.5	6.00	6.05
			12	59.6	60.2	6.10	6.10

TABLE 3—*Concluded*

SOIL NUMBER	SOIL	METHOD OF SHAKING	TIME OF SHAKING	NITRATE NITROGEN		H-ION CONCENTRATION	
				In dialysate	In filtered extract from inside of sack	In dialysate	In filtered extract from inside of sack
Average of all soils.....		Hand	hours	p.p.m.	p.p.m.	pH	pH
			4	33.1	40.4	5.79	5.78
			18	36.0	35.8	5.80	5.78
		24	35.3	36.0	5.80	5.79	
		Drawer	2	34.7	35.5	5.78	5.81
			12	36.9	36.3	5.80	5.80

* Calculations for nitrate nitrogen made on basis of dry soil.

It will be seen that with the six soils studied a complete equilibrium of nitrate ions is usually not established during the 4-hour hand shaking period. The 18- and 24-hour hand shaking periods as well as the 2 and 12 hours of drawer shaking, however, do establish complete equilibrium. Thus the superiority of the drawer shaking method in establishing equilibrium in a shorter period of time is again demonstrated. It will also be noticed that with most soils the 24-hour hand shaking method gave dialysates of slightly lower nitrate content than did the 18-hour. This is probably due to a little denitrification, for upon repeating the 24-hour period with and without the addition of 10 drops of toluene, it was found that the average content of nitrate nitrogen where toluene was added was 1.1 p.p.m. greater than where toluene had not been added.

From the data obtained with these six soils it appears that equilibrium of hydrogen ions is established in the 4-hour hand shaking period. Other soils worked with, however, did not attain equilibrium before 8 to 12 hours standing. It seems advisable, therefore, in order to be sure that equilibrium is established, to allow the flasks to stand over night. This means under most conditions a period of about 18 hours.

A comparison of the data in tables 2 and 3 shows that the diffusion of nitrate and hydrogen ions is very rapid at first. Thus as an average of the six soils, 25.3 p.p.m. nitrate nitrogen is found after 1 hour as compared to 31.5 p.p.m. after 18 hours of hand shaking; in other words, 70 per cent of the nitrate nitrogen had diffused through the sacks at the end of 1 hour when the hand method of shaking was used. With the use of drawer shaking, 87.2 per cent of the nitrate nitrogen had diffused through the collodion sack at the end of 1 hour.

From this study it is apparent that for the determinations of ions that come into solution readily as do nitrate and hydrogen ions, and possibly for such other ions as chloride, either the 2-hour drawer shaking or the 18-hour hand shaking methods can be used. The equilibrium is probably established by

TABLE 4

*The specific resistance and the phosphorus and calcium content of soil extracts after various periods of time**

SOIL NUMBER	SOIL	TIME	PHOSPHORUS		CALCIUM		SPECIFIC RESISTANCE	
			In dialy-sate	In filtered extract from inside of sack	In dialy-sate	In filtered extract from inside of sack	In dialy-sate	In filtered extract from inside of sack
		<i>hours</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>ohms</i>	<i>ohms</i>
7	Gray silt loam	6	7.6	14.2	63.7	93.8	11,410	7,160
		18	11.0	15.4	71.8	74.0	8,290	7,060
		30	13.9	17.7	88.2	80.0	7,650	7,500
		48	15.4	13.2	85.6	89.2	7,650	7,810
8	Gray silt loam	6	1.87	3.31	38.2	50.4	11,970	8,920
		18	2.78	2.93	47.0	47.0	10,790	9,160
		30	2.49	2.84	47.0	46.1	9,730	8,640
		48	2.99	3.09	47.0	8,800	11,720
9	Norfolk sandy loam	6	0.63	0.86	68.7	77.4	6,390	5,320
		18	0.84	0.92	67.8	67.8	5,660	4,810
		30	1.24	1.44	74.8	81.7	5,320	5,220
		48	1.60	1.72	85.9	85.9	5,220
2	Sumter clay	6	Trace	Trace	88.7	106.0	10,330	8,450
		18	Trace	Trace	111.0	120.5	8,880	7,960
		30	Trace	Trace	131.0	151.0	7,500	6,930
		48	Trace	Trace	145.0	139.0	6,930	6,930
6	Brown silt loam	6	0.35	0.40	67.8	113.0	8,640	5,320
		18	0.55	0.45	78.3	94.8	6,930	5,660
		30	0.45	0.40	90.4	95.6	5,770	7,770
		48	0.50	0.50	102.6	100.9	6,010	5,770
10	Norfolk sand	6	1.30	2.10	39.2	45.2	15,300	12,220
		18	1.90	2.40	38.2	44.6	14,920	13,350
		30	2.30	2.60	46.3	44.0	12,500	12,790
		48	2.75	3.00	45.2	44.0	13,060	13,060
Average of all soils....		6	1.96	3.96	61.1	81.0	10,673	7,898
		18	2.85	3.68	69.0	74.8	9,246	8,000
		30	3.39	4.16	79.6	83.1	8,078	7,808
		48	3.87	3.59	85.3	84.3	7,945	8,410

* Calculations for phosphorus and calcium made on basis of dry soil.

hand shaking in less than 18 hours, but since it seemed best to allow the flasks to stand over night, 18 hours appeared to be the most practical period of time. Thus, if the flasks are started in the afternoon, equilibrium will be established by the next morning.

Difficultly soluble constituents. With the less readily soluble constituents of the soil such as phosphorus, calcium, and potassium, the process of solution is slow. With those ions which show a considerable solubility effect, such as phosphorus, a long time is required for the establishment of equilibrium between the inside and outside solutions. In explaining this fact three factors must be considered: First, equilibrium must be established between the soil and the water inside the sack; second, equilibrium must be established between the water inside and outside of the sack; third, as diffusion takes place from the inside, the equilibrium between the soil and the water on the inside is

TABLE 5

*The specific resistance and phosphorus and calcium concentration of the dialysate after 18 hours when various proportions of water are used on inside and outside of sack**

SOIL NUMBER	SOIL	AMOUNT OF WATER		PHOSPHORUS	CALCIUM	SPECIFIC RESISTANCE
		Inside sack	Outside sack			
		cc	cc	p p.m.	p p.m.	ohms
7	Gray silt loam	200	200	11.00	71.8	8,290
		100	300	10.70	75.7	8,980
8	Gray silt loam	200	200	2.78	47.0	10,790
		100	300	2.22	39.0	10,570
9	Norfolk sandy loam	200	200	0.84	67.8	5,660
		100	300	0.72	69.6	5,770
2	Sumter clay	200	200	Trace	111.0	8,800
		100	300	Trace	113.0	8,800
6	Brown silt loam	200	200	0.55	78.3	6,930
		100	300	Trace	75.5	6,930
10	Norfolk sand	200	200	1.90	38.2	14,920
		100	300	1.50	35.3	15,300

* Calculations for phosphate and calcium made on basis of dry soil.

disturbed and as a result more material comes into solution. It is a rather complex system and a true equilibrium would be obtained only after a very long period of time. Thus, in preliminary work it was found that the amount of phosphorus and calcium in the dialysates increased slightly from the second to the third day. It would seem, therefore, that the hand method of shaking would be the most practical. To find the length of time necessary for the establishment of equilibrium of the difficultly soluble ions with the use of the hand method of shaking, the following experiment was conducted. Soil dialysates were prepared by placing 80 gm. of soil and 200 cc. of distilled water on the inside of the 500-cc. sack, and 200 cc. of water on the outside of the sack.

The shaking was done by hand as in the previous experiment with nitrate and hydrogen ions. After 4, 18, 30, and 48 hours the dialysates were removed for determinations of phosphorus, calcium, and specific resistance.

The data are reported in table 4. It will be seen that with time, there is a gradual decrease in the specific resistance and an increase in phosphorus and calcium. As is to be expected, the total diffusion of ions is large during the first six hours, and gradually decreases with time as the more difficultly soluble constituents gradually come into solution. Thus, if the average of all soils is considered it will be noticed that the increase in the amount of phosphorus in the dialysate is 45.4 per cent between the sixth and eighteenth hour, 18.9 per cent between the eighteenth and thirtieth hour, and 14.1 per cent between the thirtieth and forty-eighth hour. Similarly with calcium the increase in the second period (6 to 18 hours) is 12.0 per cent, in the third period 15.3 per cent, and in the fourth period 7.2 per cent. The same general comparison is seen in the specific resistance. It is evident, therefore, that an arbitrary time of

TABLE 6

The specific resistance, H-ion concentration and nitrate, phosphorus, and calcium content of soil extracts before and after dialyzing with new and used collodion sacks

MEMBRANE USED	EXTRACT OF SANDY LOAM SOIL					EXTRACT OF SILT LOAM SOIL				
	Specific resistance	H-ion concentration	Nitrate nitrogen	Phosphorus PO ₄	Calcium Ca	Specific resistance	H-ion concentration	Nitrate nitrogen	Phosphorus PO ₄	Calcium Ca
	ohms	pH	p.p.m.	p.p.m.	p.p.m.	ohms	pH	p.p.m.	p.p.m.	p.p.m.
None	5,170	5.15	4.60	0.11	8.3	16,100	5.35	2.61	0.09	3.9
New sack	5,159	5.10	4.66	0.07	8.2	16,280	5.35	2.62	0.09	2.9
Used sack . . .	5,148	5.10	4.56	0.09	8.2	16,100	5.35	2.62	0.08	3.1

shaking and standing of the system must be chosen. From this study and from further work considered in the latter part of this paper it seems that the 18-hour period with hand shaking (20) is the most satisfactory.

Since in certain cases large amounts of extract are desired, it becomes of practical value to know whether the proportion of water on the outside of the sack could be increased, thus giving a large amount of extract for analysis. The results of such a study are shown in table 5. The data indicate that the relative amounts of water used on the inside and outside of the sack have practically no effect on the concentration of calcium and phosphorus in the dialysate nor on the specific resistance of the dialysate after the flasks have stood for 18 hours.

Does the collodion sack absorb any of the soil constituents?

The question as to whether the sacks might absorb some of the soil constituents, or cause a contamination of the extract if they are used repeatedly, was studied, and the data are reported in table 6. Two hundred twenty

cubic centimeters of soil extract was placed on the inside of the sack, and an equal amount of water placed in the 500-cc. Erlenmeyer flask. After equilibrium had been established the dialysate was analyzed for phosphorus, nitrate nitrogen, and calcium, and its specific resistance and H-ion concentration were also determined.

It will be noticed that with the exception of phosphorus, none of the soil constituents were affected even when old sacks were used. Additional experiments with soil solutions of different phosphorus concentration, showed that frequently sacks will absorb small amounts of phosphorus. The maximum error caused by this absorption was found to be approximately as follows: for solutions containing 0.4 p.p.m. PO_4 , 10 per cent; for solutions containing 0.2 p.p.m. PO_4 , 20 per cent; for solutions containing 0.1 p.p.m. PO_4 , 25 per cent. This error is only introduced when solutions are being dialyzed. It is not a factor when soil suspensions are being dialyzed in the preparation of soil extracts. In the latter case the small amount of phosphorus absorbed by the sack is readily replaced by more phosphorus coming into solution.

COLORIMETRIC DETERMINATION OF THE H-ION CONCENTRATION

It is well established that the colorimetric method for the determination of the H-ion concentration of soils is simple, time-saving, and at the same time sufficiently accurate for most soils work. A review of the work done with the colorimetric method, and a study of the various precautions to be observed in its use were made by Pierre (21). The main precautions may be briefly summarized as follows: (a) Importance of obtaining clear extracts, (b) necessity of having neutral indicator solutions, and (c) need of protecting solutions from the laboratory air while centrifuging and after they are obtained. By following these precautions he was able to obtain good agreement between the colorimetric and electrometric methods of determining the H-ion concentration of soils. Good agreement has been obtained by other investigators, especially by Niklas and Hock (18).

The principle of dialysis through collodion membrane was used in H-ion studies as early as 1915 by Levy and coworkers (13) in the determination of the H-ion concentration of blood. More recently Kolthoff (12) compared the H-ion concentration of soil extracts obtained by dialysis through parchment membranes with that of soil extracts obtained by filtering. He made the determinations on the dialysate and filtered extract by the colorimetric method and also on the filtered extract by the electrometric method. His results, however, do not show a very good correlation between the three methods. This can be explained partly by the fact that soil extracts are usually too slightly buffered to be determined accurately by the electrometric method as commonly used.

In the present study the authors determined the H-ion concentration of soil extracts, obtained both by dialysis and by suction filtering, by means of the colorimetric method. The H-ion concentrations of the soil suspensions were

also determined by the electrometric method. The dialyzed extract was obtained according to the method previously recommended for nitrates and H-ion determination, that is, allowing the flasks to stand 18 hours with occasional shaking by hand.

The dialysate and filtered extract were in most cases obtained from the same flask, the soil suspension inside the sack being filtered, by the modified suction method previously described, to give the extract referred to in table 7. The electrometric determinations were made on 1:2 soil-water suspensions which had been shaken for one-half hour. A Leeds and Northrup improved type of potentiometer and sensitive enclosed lamp and scale galvanometer were used. The data obtained (table 7) show a good correlation among the three methods.

The collodion sack method has also been found very useful in determining the buffer capacity of soils. In brief the method for buffer studies consists in preparing a series of flasks, using, for example, 40 gm. of soil to 80 cc. of water. To the flasks are then added varying amounts of some acid or base, determined by the range in which the buffer capacity of the soil is desired. Barium hydroxide and hydrochloric acid were used by the writers. The flasks are then allowed to stand with occasional shaking until equilibrium is established between the soil and the acid or base added. This can be readily determined by making H-ion determinations on 5 cc. of the dialysate at intervals until there is no longer any change during a period of 24 hours. This will usually be after 48 hours. If the amount of acid or base added to the soil and the H-ion concentration of the dialysates at equilibrium are known, the buffer capacity of the soil can be readily calculated.

In a brief study of the use of parchment shells for obtaining soil dialysates for H-ion determinations it was found that with the four soils studied, new shells gave extracts of a H-ion concentration equal to that of extracts obtained by the use of the collodion sack. It was found, however, that the shells may absorb bases and acids from the soils and that these bases and acids cannot be washed out readily. Consequently, if shells were used more than once with soils of quite different H-ion concentration they were found to give up some of the bases or acids previously absorbed and to give dialysates of high or low H-ion concentration. On the other hand, collodion membranes used repeatedly with soils of different reactions showed no absorption effects and gave extracts of the same H-ion concentration as those obtained with new sacks.

The collodion sack method, therefore, is of special value in obtaining soil extracts for the determination of the H-ion concentration of soils by the colorimetric method. Although the suction filtering method makes possible the securing of extracts in a shorter time, the collodion sack method is much more rapid if a large number of extracts are to be obtained. Thus, if twenty soil extracts are to be prepared, the flasks with sacks can be set up in a period of about 20 minutes, shaken a few times that afternoon, and the H-ion determinations made by the colorimetric method on the dialysate the next

morning in less than half an hour. Moreover, the collodion sack method offers fewer difficulties in the securing of clear soil extracts than does the suction filtering method. As was shown by one of the authors (21) the latter method requires that care be taken in washing the filter paper, and in preventing too much suction when soils of low H-ion concentration are filtered.

DETERMINATION OF NITRATES

The phenoldisulfonic acid method is commonly used for the determination of nitrate in soils. It is simple, rapid, and sufficiently accurate for all practical purposes, provided a few precautions are followed. Probably the most important of these precautions is the securing of a clear and colorless extract. In order to get clear extracts various methods of obtaining soil extracts have

TABLE 7

The H-ion concentration of soil extracts, obtained by dialysis and by filtration, as compared with that of soil suspensions

SOIL NUMBER	SOIL	H-ION CONCENTRATION OF SUSPENSIONS (ELECTROMETRIC)	H-ION CONCENTRATION OF DIALYSATE (COLORIMETRIC)	H-ION CONCENTRATION OF FILTERED EXTRACT (COLORIMETRIC)
		pH	pH	pH
1	Norfolk sandy loam	4.85	4.95	4.85
12	Decatur silt loam	5.17	5.15	5.10
16	Gray silt loam	7.10	7.10	7.00
17	Norfolk sandy loam	4.92	5.00	5.00
18	Norfolk sandy loam	5.87	6.00	6.00
19	Norfolk fine sand	6.42	6.35	6.40
20	Decatur silt loam	5.61	5.60	5.60
21	Wooster silt loam	6.66	6.85	6.80
22	Ruston sandy loam	5.11	5.15	5.00
23	Norfolk sandy loam	5.99	6.05	6.00
24	Houston clay	7.88	7.80	7.80
25	Decatur silt loam	4.46	4.55	4.50

been used, but the use of flocculating agents with subsequent filtering seems to have become the most general procedure. Harper (10) has recently made a study of the method and gives a good review of the literature. After making a comparison of various flocculents used, he recommends the use of a combination of copper sulfate, calcium oxide, and magnesium carbonate. The collodion sack method gives clear extracts and seems well adapted for use in nitrate determinations. It seemed desirable, therefore, to compare this method with other methods of obtaining extracts for nitrate determinations.

In table 8 is given the nitrate content of soil extracts as determined by the phenoldisulfonic acid method when the extracts had been obtained by different methods. The figures given are averages of duplicate determinations. The flocculation method used was that recommended by Harper, and the dialysates

were obtained as previously recommended. With the suction filtering method the suspensions were prepared according to the procedure of Stewart (25). Filtration was done with the modified suction filtering apparatus previously mentioned.

As the data indicate, the agreement among the three methods is in most cases good. The flocculation method, however, gives erratic results in a few cases. Upon making duplicate determinations of nitrates on these extracts the flocculation method often gave poorer checks than did the extracts from the other two methods. This was believed to be due partly to the large

TABLE 8
Nitrate nitrogen in soils when the extracts are obtained by different methods

SOIL NUMBER	SOIL	METHOD OF OBTAINING SOIL EXTRACTS		
		Dialysis	Modified suction filtering	Flocculation by Harper method
		<i>p p m.</i>	<i>p p m.</i>	<i>p p m.</i>
1	Norfolk sandy loam	66 2	67 4	73.4
2	Sumter clay	5.4	6 5	6.6
3	Gray silt loam	8 6	9.9	9 5
4	Greenville sandy loam	48 7	49 3	42.5
5	Dekalb fine sandy loam	25 9	27 2	24.1
6	Brown silt loam	65 7	66 9	73.5
11	Dekalb fine sandy loam	6.3	6 3	7.2
12	Decatur silt loam	10.8	9.9	10.6
13	Greenville sandy loam	42.6	43.2	41.2
14	Greenville sandy loam	4.6	4.7	5.4
15	Black silt loam	25.8	25 7	28.6
	Average	28.2	29 7	29.3

amount of salts present as a result of the reagents added, and partly to the fact that there was often a slight interfering color in these extracts.

The use of flocculents was also found to necessitate the making of a nitrate determination on the reagents. Thus, it was found that c. p. magnesium carbonate contained appreciable amounts of nitrates. On the other hand, certain kinds of filter paper may absorb nitrates, as was shown by Gimingham and Carter (9). The collodion sack method eliminates both of these sources of error, and insures the securing of clear extracts.

The use of decolorizing agents

Beside the slight turbidity of extracts, which is commonly present and which interferes in the phenoldisulfonic acid method of determining nitrates, there is also, with some extracts, the interference of color due to the presence of soluble organic matter. This is usually overcome by the addition of absorbents. As is obvious, absorbents can be used equally well with the collodion sack method as with any other method.

A review of the literature indicates that carbon black and alumina cream are the absorbents most commonly recommended. Chamot and coworkers (4), and later Emerson (7) recommended the use of alumina cream, whereas Schreiner and Failyer (23), Harper (10) and others found "G Elf" carbon black to be satisfactory. In a brief comparison the writers found that carbon black was much superior to alumina cream in decolorizing power. It was also found that alumina cream absorbed appreciable amounts of nitrates. Harper, however, raised the following objections to the general use of carbon black; (a) the passing of finely divided carbon through the filter paper which caused a black residue upon evaporation, (b) the possibility of the carbon black absorbing more or less nitrate, (c) the inability of carbon black to flocculate soil suspensions, and (d) the difficulty of removing it from the apparatus. It will be noticed that the first and third objections are removed when the collodion sack method is used. It was also found that the sacks could readily be cleaned practically free of carbon black.

TABLE 9

Nitrate nitrogen in soils when various kinds and various amounts of carbon black are added to the soil suspension in the collodion sack

SOIL NUMBER	SOIL	TREATMENT WITH CARBON BLACK				
		None	0.2 gm. "Pfanstichl"	0.5 gm. "Pfanstichl"	0.2 gm. "G Elf"	0.5 gm. "G Elf"
		<i>p p m.</i>	<i>p p m.</i>	<i>p p m.</i>	<i>p p m.</i>	<i>p p m.</i>
3	Gray silt loam	8.3	7.2	5.0	8.2	8.4
6	Brown silt loam	64.6	62.6	60.5	62.2	64.8

A brief study was made of the possibility of absorption of nitrates by two different kinds of carbon black. The carbon black was added to the soil inside the sack (20 gm. of soil) and the nitrates were determined in the dialysate after the usual 18 hours standing. The results are presented in table 9. It is evident that the "Pfanstichl" decolorizing carbon will absorb nitrates from some soil extracts if used in large amounts, while the "G Elf" has no effect.

In working with a compost soil giving a highly colored extract 0.2 gm. of carbon black added to 20 gm. of soil was found sufficient to decolorize the extract. Therefore, in working with soils giving colored extracts it is recommended that 0.2 gm. of "G Elf" carbon black be added to the soil inside the sack.

PHOSPHORUS AND CALCIUM DETERMINATIONS

The collodion sack method of obtaining soil extracts was used with advantage by Parker and Tidmore (20) in the determination of phosphorus in the soil extract. They allowed 18 hours for solution and diffusion to take place before they analyzed the dialysate. Since the solution of soil phosphorus, calcium,

and other mineral soil elements is very gradual and continuous, as was pointed out in the equilibrium studies in table 4, an arbitrary period of time for solution and diffusion must be selected. Table 4 indicates that 18 hours is a very satisfactory period to use. It then seemed desirable to compare this procedure with other methods of securing extracts.

Table 10 gives the concentration of phosphorus and calcium and the specific resistance of soil extracts obtained by the collodion sack method, the modified suction filter method, and the Pasteur-Chamberland filter methods. In the latter two methods the soil suspension was given the preliminary mixing used

TABLE 10
*The specific resistance and the phosphorus and calcium concentration of the soil extracts obtained by different methods**

SOIL NUMBER	SOIL	METHOD	PHOSPHORUS	CALCIUM	SPECIFIC RESISTANCE
			<i>p.p.m.*</i>	<i>p.p.m.</i>	<i>ohms</i>
7	Gray silt loam	Collodion sack	12.10	67.8	8,120
		Suction filter	13.40	76.5	7,060
		P. C. filter	9.90	67.0	7,960
8	Gray silt loam	Collodion sack	3.10	40.0	10,100
		Suction filter	3.40	40.8	9,160
		P. C. filter	2.70	33.9	9,740
9	Norfolk sandy loam	Collodion sack	1.00	71.3	5,220
		Suction filter	1.00	74.8	5,220
		P. C. filter	0.50	74.8	5,220
6	Brown silt loam	Collodion sack	0.75	68.7	6,640
		Suction filter	Trace	76.5	8,120
		P. C. filter	Trace	62.6	8,450
10	Norfolk sand	Collodion sack	2.10	40.0	13,960
		Suction filter	2.20	40.8	14,620
		P. C. filter	1.40	33.0	16,390

* Calculations made on basis of dry soil.

by Stewart (25) in his soil extract studies. One-half the suspension was filtered through a Pasteur-Chamberland filter and the other half through the modified suction filter. The dialysate was obtained after 18 hours, the hand method of shaking being used. The phosphorus was determined by the Coerulomolybdate colorimetric method used by Parker and Tidmore, and the calcium was determined by the ordinary volumetric method. Since in most cases only 100 cc. of extract was used for calcium determinations, the minor discrepancies noticed in the table can be accounted for.

The data in table 10 show that the collodion sack method and the suction filter method give extracts of practically the same concentrations of phosphorus

and calcium. It is evident, therefore, that the 18-hour dialysis gives as good an arbitrary method of obtaining soil extracts for the study of the water-soluble mineral constituents of the soil as do the ordinary filtering methods. As is again shown, the Pasteur-Chamberland filters may absorb appreciable amounts of phosphorus.

It is evident from these studies that the collodion sack method is not only adaptable to the securing of extracts for determinations of H-ion concentration, of nitrates, and of water-soluble phosphorus and calcium, but also that it should be equally well adapted for use when any of the other water-soluble constituents of the soil are to be determined. Not only does it furnish large amounts of clear extract from which all water-soluble constituents can be determined by even the most exacting colorimetric methods, but also its use is simple. Moreover, as used by Parker and Tidmore (20) it furnishes a convenient method of studying the solubility of soil constituents.

SUMMARY

The advantages and disadvantages of different methods of obtaining soil extracts are considered. The use of collodion sacks in obtaining soil extracts by dialysis seems to offer a method that is short and simple and gives the soil extract in an unaltered condition. The method was studied in detail.

1. By a procedure given in detail, twenty collodion sacks can be made easily in one hour.

2. A sack may be used several times in preparing soil extracts. Neither old nor new sacks influence the composition of the extract.

3. Equilibrium studies on the dialysate and on the filtered soil suspension from the inside of the sacks, obtained after various periods of contact and with various methods of shaking of the system, may be summarized as follows:

- a. Continuous mechanical shaking of the dialyzing system or flasks for 1 hour gave a quicker diffusion of ions through the membrane than did hand shaking at intervals of 15 minutes for the same period.

- b. For ions that come into solution quickly, 2 hours of shaking of the flasks in a drawer type shaker, or 18 hours standing with occasional hand shaking was found to establish complete equilibrium. Both of these methods of shaking are recommended as practical.

- c. For ions that come into solution slowly, the arbitrary time of 18 hours of standing, with hand shaking of the flasks once every hour during the day, is recommended. This procedure was found to give extracts of practically the same concentration of phosphorus and calcium as does the common method of obtaining extracts.

4. A study of the collodion sack method of obtaining soil extracts for the determination of nitrates, H-ion concentration, and water-soluble phosphorus and calcium established the following points:

- a. The H-ion concentrations of soil extracts obtained by the collodion sack method, of filtered soil extracts determined by the colorimetric method, and of soil suspensions determined by the electrometric method agree well. The collodion sack method, therefore, is of special value where the H-ion concentration of soils is to be determined by the colorimetric method.

- b. The buffer capacity of soils can be determined readily by means of the "Dialysis-colorimetric" method.

c. Extracts obtained by the collodion sack method have the same concentration of nitrates as do extracts obtained by filtering or flocculation methods.

d. "G Elf" carbon black completely removes the color of extracts due to soluble organic matter without affecting the nitrate nitrogen content of the extract.

e. The collodion sack method not only offers a new simple method for use in the determination of the water-soluble constituents of the soil but also offers a means of studying the solubility of the relatively insoluble soil constituents.

5. The advantages of the collodion sack method over other methods of obtaining soil extracts may be briefly summarized as follows:

a. It gives clearer soil extracts than is usually obtained by other methods, and is, therefore, especially adapted to the determination of the water-soluble constituents of the soil by colorimetric methods.

b. It does not affect the constituents of the soil extract as does filtering with Pasteur-Chamberland filters and in some cases with filter paper.

c. It requires no flocculating agents, and thus eliminates any error due to the addition of flocculents.

d. It requires little apparatus, is simple and inexpensive, and is well adapted to routine laboratory work where large numbers of extracts are to be obtained.

REFERENCES

- (1) BIGELOW, S. L., AND GEMERLING, A. J. 1907 Collodion membranes. *Jour. Amer. Chem. Soc.* 29: 1576.
- (2) BROWN, W. 1915 The preparation of collodion membranes of differential permeability. *Biochem. Jour.* 9: 591-617.
- (3) BROWNE, R. J. 1923 The ultra filtration of tannin and other solutions. *Jour. Amer. Leather Chem. Assoc.* 18: 409-23.
- (4) CHAMOT, E. M., PRAT, D. S., AND REDFIELD, H. W. 1910 A study of the phenol-sulphonic acid method for the determination of nitrates in water. *Jour. Amer. Chem. Soc.* 33: 366-384.
- (5) DENISON, IRVING A. 1922 The nature of certain aluminum salts in the soil and their influence on ammonification and nitrification. *Soil Sci.* 13: 81-106.
- (6) EGGERTH, ARNOLD H. 1921 The preparation and standarization of collodion membranes. *Jour. Biol. Chem.* 48: 203-21.
- (7) EMERSON, PAUL 1921 The colorimetric determination of soil in a colored water extract. *Soil Sci.* 12: 413-417.
- (8) FARMER, CHESTER J. 1917 A method for the preparation of uniform collodion membranes for dialysis. *Jour. Biol. Chem.* 32: 447-453.
- (9) GIMINGHAM, C. T., AND CARTER, R. H. 1923 On the estimation of nitrates in soils by the phenoldisulphonic acid method. *Jour. Agr. Sci. [England]* 13: 60-62.
- (10) HARPER, HORACE J. 1924 The accurate determination of nitrates in soils. *Jour. Indus. and Engin. Chem.* 16: 180-183.
- (11) KNIGHT, H. G. 1920 Acidimetry and alkalinity of soils. *Jour. Indus. and Engin. Chem.* 12: 340-344.
- (12) KOLTHOFF, I. M. 1923 The colorimetric determination of the hydrogen exponent of the soil. *Chem. Weekbl.* 20: 675-677.
- (13) LEVY, R. L., ROWNTREE, L. G., AND MARRIOTT, W. MCK. 1915 A simple method for determining variations in the hydrogen-ion concentration of the blood. *Arch. Int. Med.* 16: 389-405.
- (14) LOEB, JACQUES. 1924 Proteins and the Theory of Colloidal Behavior, ed. 2. New York.
- (15) MAGISTAD, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181-226.

- (16) MIRASOL, J. J. 1920 Aluminum as a factor in soil acidity. *Soil Sci.* 10: 153-217.
- (17) NELSON, J. M., AND MORGAN, JR., D. P. 1923 Collodion membranes of high permeability. *Jour. Biol. Chem.* 58: 305-319.
- (18) NIKLAS, H., AND HOCK, A. 1919 Determination of the H-ion concentration of soils by the colorimetric method. *Ztschr. Angew. Chem.* 38: 150.
- (19) PARKER, F. W. 1925 The absorption of phosphate by Pasteur-Chamberland filters. *Soil Sci.* 20: 149-158.
- (20) PARKER, F. W., AND TIDMORE, J. W. 1926 The influence of lime and phosphatic fertilizers on the phosphorous content of the soil solution and of soil extract. *Soil Sci.* 21: 425-441.
- (21) PIERRE, W. H. 1925 The H-ion concentration of soils as affected by carbonic acid and the soil-water ratio, and the nature of soil acidity as revealed by these studies. *Soil Sci.* 20: 285-305.
- (22) SCHOEF, A. 1911 Filtration of colloidal solutions. A new filter. *Bul. Soc. Chim. Belg.* 24: 354-67.
- (23) SCHREINER, O., AND FAIRLYER, G. H. 1906 Colorimetric, turbidity, and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31.
- (24) SHAW, JAC. A. 1917 A new filter flask. *Jour. Indus. Engin. Chem.* 9: 793.
- (25) STEWART, GUY W. 1918 Effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12: 311-368.
- (26) TRUOG, EMIL 1925 The colloid chemistry of soils. *Colloid Symposium Monog.* 3: 228-240.

THE OCCURRENCE OF YEASTS IN SOIL

R. L. STARKEY AND A. T. HENRICI¹

University of Minnesota

Received for publication April 23, 1926

INTRODUCTION

Besides the numerous bacteria and actinomyces and a few molds that develop upon the plate from soil, there may occur occasionally a colony of a yeast. Plates for counting bacteria and actinomyces, however, are generally made at relatively high dilutions of soil (100,000 to 10,000,000) and it would not appear surprising if yeasts failed to appear in abundance at these dilutions even though they were numerous in the soil.

The general occurrence of yeasts in considerable abundance on fruits and flowers suggests their presence in soils. That they do occur there has been repeatedly shown, but in what abundance may they be expected? Previous studies on the occurrence of yeasts in nature give little information on the abundance with which they may be found in soil or what species may be most frequently encountered. There appears to have been no systematic study conducted to determine whether they occur sufficiently regularly or abundantly to be factors of importance in soil transformations, whether they should be considered normal soil inhabitants or occasional invaders.

The following studies were conducted to cast light upon the presence of yeasts in soil, to determine how widely they may be found, in how great a diversity of forms, and in what abundance. It was of particular interest to determine whether yeasts might be considered as important elements of the microscopic population of the soil.

HISTORICAL

The extensive researches of Hansen on yeasts embraced studies on the cycle of some yeasts in nature, particularly *Saccharomyces (Hansenia) apiculatus* and some of the true *Saccharomycetes*. In the early studies on the apiculate yeasts, Hansen (12, 13) determined that these forms depended principally upon the sweet, ripe, juicy fruit for their proliferation. Developing on the fruit they would become disseminated principally by air currents and also by insects and rainfall to other fruits and soil. The fallen fruit also carried yeasts to the soil. They could persist for some time in soil but apparently did not find conditions particularly favorable for development. It was believed that they lived over the cold months in soil and were introduced upon fruit again from soil by dust, insects, and other natural agencies. Accordingly it was noted that these yeasts were more abundant during the fruiting season and less numerous in the spring. Their numbers also increased in the atmosphere during the late

¹ Department of Bacteriology and Immunology.

summer (14). When yeasts (*Saccharomyces apiculatus*, *S. pastorianus*, *S. ellipsoideus*, Carlsberg No. 1, and a brewery yeast) were inoculated into soil and kept under natural conditions they persisted for at least a year although their numbers decreased (15). *S. apiculatus* was still found in the inoculated soil after exposure to natural conditions for three years.

It was later shown (16, 17, 18) that the true *Saccharomyces* pass through a cycle much the same as the apiculate yeasts, but because the true *Saccharomyces* form spores and *S. apiculatus* does not, the former persisted longer and appeared more widely distributed. Since the most important region of development of these yeasts is fruit, it is not surprising that they were found more abundantly in soils of vineyards and orchards and less frequently at distances from these regions. *Saccharomyces* were found in 67 per cent of samples of soil under fruit trees, in 30 per cent of samples of soil in neighboring woods, and in 19 per cent of samples in remote fields. Soils of mountainous regions contained extremely few *Saccharomyces*. The true *Saccharomyces* were found more widely distributed than the apiculate yeasts, and *Willia* and *Pichia* forms were more tolerant to natural climatic conditions than the true *Saccharomyces*. Although these data suggest that these forms may occur temporarily in soil they do not indicate that yeasts are of importance in soil transformations.

Many of these observations were verified or amplified by Berlese (2), Muller-Thurgau (29, 30), Wortmann (37), Cordier (7), Klocker (22), Boutroux (3, 4, 5), and Heinze (19). [See also Klöcker (23) and Guilliermond (11).]

Muller-Thurgau (29) determined that wine yeasts occurred in orchard and vineyard soils not only at the surface but even at 20 to 30 cm. below the surface. None were found at a depth of 40 cm. Berlese (2) found them in vineyards and orchards down to 12 or 13 cm.

Fischer (9) found that yeasts were often favored by soils rich in humus and found them particularly abundant in high moor soils. Klöcker (22, 21, 25, 24) isolated many new species of yeasts from soils from various parts of the world. These included *Saccharomyces*, *Endomyces*, *Debaromyces*, and *Schwannomyces*. Ludwig (27), DeKruyff (8), and Adametz (1) obtained yeasts from soil.

Pasteur early showed that yeasts did not occur abundantly on green fruit (32, 35, 31) but developed rapidly on the ripened fruit if no precautions were taken to prevent their deposition on the fruit. Yeasts have been found in considerable abundance in flowers by Boutroux (3, 4, 5), Berlese (2), Cordier (7), Schuster and Vladimir (34), and Reukauf (33). Several of these authors have verified Pasteur's observations on the absence of yeasts on immature fruit.

That insects may be important factors in transporting yeasts from fruit to fruit and from flower to flower is quite generally accepted (17, 29, 37, 22, 3, 5, 4). In these instances the yeasts may be merely carried on the surfaces of the insects' bodies or pass through their digestive systems. Brefeld (6) and Berlese (2) considered that yeasts might not only multiply in the digestive tracts of some insects, but might have their principal abode there.

As regards the occurrence of *Torulae* in soil there appears to be very scant information. Adametz (1) found white as well as red species widely distributed. DeKruyff (8) found that the *Torulae* composed a comparatively small proportion of the total number of yeasts found in soil and foliage. Ludwig (27) isolated some *Torulae* from soils of the Alps. Klöcker (22) cites Hansen as finding species of *Torula* distributed widely in nature. Kohl (26) states that the greatest number of yeasts which are found in nature on fruit or in soil are wild yeasts.

Most of these researches appear to have been primarily concerned with the sources of yeasts that occur on fruit and not with the importance of yeasts as representatives of the soil flora.

METHODS

For obtaining the soil samples, sterile cylindrical samplers were used (10.5 cm. long by 2 cm. inside diameter). Samples were taken to a depth of 6½

inches after the litter on the soil surface was removed. The soil was removed from the samplers by sterile wooden plungers to sterile 250-cc. bottles. These samples, which weighed from 20 to 50 gm., were suspended in 100 cc. of sterile tap water. One-cubic centimeter portions of these suspensions (equivalent to 0.2 or 0.3 gm. of soil) were inoculated into 100 cc. of a medium composed of 90 cc. nutrient broth and 10 cc. dextrose-tartaric acid solution. The dextrose-tartaric acid solution was composed of 50 per cent dextrose and 5 per cent tartaric acid. This solution was sterilized separately from the broth and added after sterilization. The final reaction of the medium was close to pH 3.8. This acidity prevented the development of bacteria but supported growth of yeasts and filamentous fungi. The molds tended to form a heavy pellicle upon the surface. In order to obtain samples relatively free of molds from these cultures, flasks were used which had a tap sealed in at the bottom. If yeasts developed they could be obtained readily in samples drawn off through this tap after incubation for about a week. After the incubation period the samples were diluted and plated upon an agar medium composed of 9 cc. nutrient agar and 1 cc. of the above mentioned dextrose-tartaric acid solution. Subsequent to incubation for 24 or 48 hours, the plates were examined for yeast colonies which were planted upon Sabouraud's agar of the following composition:

	<i>per cent</i>
Peptone.....	1
Maltose.....	4
Agar.....	1.8

The cultures so obtained were studied morphologically and culturally for identification.

As alternative media for initial enrichment and plating, the medium suggested by Waksman (36) for plating filamentous fungi proved as satisfactory as the above. The solution medium was of the following composition:

	<i>per cent</i>
Dextrose.....	1
Peptone.....	0.5
KH ₂ PO ₄	0.1
MgSO ₄	0.05

About 10 cc. sterile 1/15 *N* H₂SO₄ was added to 90 cc. of this sterilized medium to bring the pH to 3.8. A solid medium was prepared containing 3 per cent agar. Just before pouring the plates 1 cc. of sterile 1/15 *N* H₂SO₄ was added to each 10 cc.

In some instances soil samples were plated directly upon the acidulated agar without previous enrichment in the solutions, and yeast colonies were picked after incubation. In some instances 100-gm. portions of soil were incubated after the addition of 1 or 2 gm. of dextrose. Later these were either plated or enriched in the nutrient-dextrose-tartaric acid solutions.

RESULTS

In all, 87 different soils, obtained principally from a region within a radius of 50 miles of Minneapolis, Minnesota, were studied. Of these, 59 were obtained from Minnesota, 9 from Wisconsin, 16 from New Jersey,² and 3 from New Hampshire. Included among these were sands, peats, clays, and muck supporting such a variety of vegetation as brush and trees, grasses, grain, garden truck, and fruit.

It was strikingly apparent throughout the studies that the soils harbored extremely few yeasts. Of the 87 soils studied, 48 (55 per cent) yielded no yeasts by any of the methods applied, whereas 39 (45 per cent) gave one or more species. Only 2 soil samples, 1 lawn and 1 garden, yielded three different species of yeast each. From 10 samples of soil two different species of yeast were obtained.

From no single sample of soil were more than three different species of yeast obtained, although several different methods were used to obtain them.

Molds occurred in abundance from most soils whether the soils were enriched in the acidulated sugar medium before plating or whether the soil was plated directly. It was particularly striking that the molds occurred in great abundance, whereas the yeasts were scant. In two cases where the yeasts appeared fully as abundantly as in any soil there occurred 26,000 and 7,600 molds per gram, whereas only a few colonies of yeasts were found. In this instance suspensions equivalent to $1/4$ or $1/40$ gm. of soil were plated for the yeasts. Dilutions of over $1/100$ gm. of the original soil would scarcely ever show yeast colonies when plated or inoculated into liquid media. Even when a suspension equivalent to $1/10$ gm. of soil was used in direct plating there seldom developed over five colonies of yeasts. Plate counts of yeasts should, moreover, give fairly good representations of the abundance of yeasts due to the type of their reproduction. These results would appear to indicate definitely the relative scarcity of yeasts in soil.

Because the yeasts appeared in such small numbers in any of the soils used, even including those supporting large and small fruit stock, there should hardly be very much importance attached to correlations between the finding of yeasts and the vegetation, reaction, soil type, or seasonal variation except as such data might show a haphazard distribution under the various conditions or a consistent presence or absence under some conditions. The following tables indicate the instances where yeasts were obtained and where they were not found.

* The authors are very grateful to Dr. Waksman of the New Jersey Agriculture Experiment Station at New Brunswick and to Mr. Jones of the experimental cranberry laboratories at Whitesbog, New Jersey, for providing soils growing cranberries and blueberries. Thanks are also extended to Prof. Blair for permission to obtain samples from the experimental plots at the New Jersey station and to Professor Rost for permission to obtain samples from the experimental plots at the University of Minnesota.

Table 1 shows a correlation between the occurrence of yeasts in soil and the plant growth. Of the 45 cultivated soils, 17 (38 per cent) gave cultures of yeasts. There appears to be no significant difference whether these soils grew legumes or non-legumes, grasses or garden truck. The proportion of soils

TABLE 1
Correlation between the occurrence of yeasts in soil and the vegetation on these soils

NUMBER OF SOILS	VEGETATION	NUMBER OF YEASTS FOUND	NUMBER OF SOILS WITH ONLY ONE YEAST	NUMBER OF SOILS WITH MORE THAN ONE YEAST	PER CENT OF SOILS WITH YEAST
45	<i>Cultivated</i>	22	13	4	38
33	Grasses and grain	13	11	1	36
6	Legumes	2	2	0	33
5	Mangels and potatoes	5	0	2	40
1	Gardens	2	0	1	100
3	<i>Lawns</i>	7	0	3	100
5	<i>Pasture and prairie</i>	2	2	0	40
19	<i>Orchards and small fruits</i>	13	7	3	53
9	Large fruits	5	1	2	33
4	Berries and grapes	2	2	0	50
2	Blueberries	2	0	1	50
4	Cranberries	4	4	0	100
8	<i>Wooded Land</i>	3	1	1	25
3	<i>Sand Dunes and Banks</i>	3	3	0	100
4	<i>Swamps and Marshes</i>	3	1	1	50
87		53	27(31%)	12(14%)	45

TABLE 2
Correlation between soil type and the occurrence of yeast

SOIL TYPE	SOILS WITH YEASTS		SOILS WITHOUT YEAST		TOTAL NUMBER OF SOILS
		<i>per cent</i>		<i>per cent</i>	
Muck.....	0	0	2	100	2
Peat.....	4	80	1	20	5
Sand.....	6	67	3	33	9
Sandy loam.....	13	38	21	62	34
Clay loam.....	11	35	20	65	31
Clay.....	5	83	1	17	6
Totals.....	39	45	48	55	87

showing yeasts was nearly the same in all instances. Included in this group of soils were 9 samples from experimental plots of New Jersey, 2 of which gave yeasts, and 9 samples from experimental plots of Minnesota, none of which gave yeast development.

The three lawn soils each gave more than one yeast, a result which appears suggestive in spite of the fact that the data are too scant to carry much weight.

Only 2 of 5 soils of pastures and prairie types gave yeasts, whereas practically half of the orchard soils gave yeasts. This low proportion was rather unexpected in the light of the results of other workers, particularly since many of these soils were studied during September, a period when yeasts should occur in these regions in the greatest abundance. The percentage of soils growing

TABLE 3
Correlation between reaction of soils and occurrence of yeasts

SOIL REACTION <i>pH</i>	SOILS WITH YEASTS		SOILS WITHOUT YEASTS		TOTAL NUMBER OF SOILS
		<i>per cent</i>		<i>per cent</i>	
4.0-4.5	4	100	0	0	4
4.6-5.0	1	50	1	50	2
5.1-5.5	2	40	3	60	5
5.6-6.0	0	0	7	100	7
6.1-6.5	9	50	9	50	18
6.6-7.0	10	45	12	55	22
7.1-7.5	1	20	4	80	5
7.6-8.1	4	44	5	56	9
Totals.....	31	43	41	57	72

TABLE 4
Correlation between season of year and occurrence of yeasts in soil

MONTH	SOILS WITH YEASTS		SOILS WITHOUT YEASTS		TOTAL NUMBER OF SOILS
		<i>per cent</i>		<i>per cent</i>	
May.....	6	24	19	76	25
July.....	1	33	2	67	3
August.....	6	40	9	60	15
September....	9	43	12	57	21
December.....	5	22	18	78	23
Totals.....	27	31	60	69	87

large fruits which showed yeasts (33 per cent) was much below that of the soils growing cranberries (100 per cent). The reaction of the last mentioned soils was considerably below that of the soils growing large fruits. Only 2 of 8 soils of woodland showed yeasts. Each of the three samples of sands developed yeasts. Only 2 of 4 samples from swamps and marshes showed yeast development.

Table 2 presents a correlation between the occurrence of yeasts and the type of soil roughly classified under six types. Yeasts were found in practically

all soils except muck. In this instance but two samples were studied, consequently these results would hardly be significant. In peat and clay a particularly high proportion of the samples yielded yeasts (80 and 83 per cent respectively). Sands also gave a high proportion. The results as a whole, however, show little that could be taken as indicative that yeasts would appear more abundantly in soils of one type than another.

Even though yeasts were found between pH 4.0 and 4.5 in all of the four soils and between pH 6.0 and 7.0 in only 60 per cent of the cases, there was no regularity in the results to indicate that the abundance of yeasts might be affected by alkaline or acid conditions. (See table 3.)

Table 4 indicates that yeasts might be found more commonly during the late summer than during the early summer or the winter.

The results as a whole indicate that the yeast population is in general so small that the effects of plant growth, soil structure, soil reaction, or seasonal variations have slight significance. The differences which appear in these results are more like chance scattering than distribution significantly correlated with environmental factors. The conclusion seems justifiable that yeasts occur in most soils in such small numbers as to be of little importance in the transformations that occur there.

DESCRIPTION OF SPECIES

The classification and identification of yeasts are not easy. This is more particularly true of the species, for most strains may be readily placed in their proper genus after a little study; but the published descriptions of species are frequently so incomplete, or their identification is based upon such variable characters, that it is almost impossible to decide whether an organism is new or one that has been previously described. It has been possible to identify positively but three of the twelve species isolated—*Nadsonia fulvescens*, *Willia anomala*, and *Torula glutinis*.

Several of the organisms isolated proved on further study not to be true yeasts, the yeast-like cells which were found on agar plates later developing mycelium. The authors were thus misled three times by the sporidia of *Ustilago zeae*; and once each by an organism closely resembling *Oidium lactis* and another very similar to *Monilia candida*, the latter producing mycelium only under semianaerobic conditions in agar stab cultures. *Chlamydomucors*, producing budding yeast-like cells and an alcoholic fermentation, also proved misleading at first.

The forty-nine strains of yeasts isolated have been observed in cultures grown on Sabouraud agar, and in large tubes of 5 per cent dextrose broth. Further study of fermentation was made in small tubes containing 1 per cent sugar broths with gas traps and Andrade's indicator; dextrose, lactose, sucrose, maltose and mannite were used. Each strain was tried three times for gelatine liquefaction, once in ordinary gelatine stabs, once in 5 per cent dextrose gelatine stabs, and once by the method recently described by Frazier (10); in no

case, however, was any digestion of the gelatine shown. All cultures were incubated at about 30°.

Morphological characters were determined from young (24-hour) Sabouraud agar cultures, and from the same cultures after they were a week or more old. For spore formation, the classical plasterblock method was used, and, with much more success, the carrot infusion calcium sulfate agar recently described by McKelvey (28). Spores were also found in old agar cultures in several instances. Morphological characters have been determined almost entirely by the examination of unstained wet preparations. The identification of spores has been confirmed in every instance by the use of Moeller's spore stain, and fat globules have been identified by the use of Sudan III and osmic acid.

It has been impossible positively to identify most of the species. However, it has appeared particularly striking that the various species maintained with marked constancy even slight differences in morphological and cultural characters over relatively long periods of subcultivation; in particular, differences in morphological characters consistently correlated with cultural characters. The twelve species were first separated almost entirely on morphological characters, but subsequent tabulation of cultural characters showed that those which were identified as the same by morphological characters had, with few exceptions, the same cultural characters.

In grouping the species isolated, the classification and nomenclature of Guilliermond (11) has been followed. The twelve species are described below, and are illustrated in the accompanying plate. The various figures are all camera lucida drawings at the same magnification. In figures 1 to 8, inclusive, the upper half shows the morphology in old cultures or cultures from McKelvey's medium for spores; the lower half shows the cells in young Sabouraud agar cultures. In the remaining figures the entire field shows cells from a young culture, the old cultures not showing any noteworthy difference.

Zygosaccharomyces sp. (fig. 1). This yeast, found but once, was the only species isolated showing definite evidence of conjugation preceding spore formation. The vegetative cells are oval and of medium size. Spores were formed readily on plaster blocks, on McKelvey's medium, and in old Sabouraud agar cultures. Practically all of the asci were of dumbbell shape, showing that conjugation had taken place; but one of the gametes was almost invariably smaller than the other. The conjugation is therefore heterogamic. From two to four spores are formed, three being the most frequent number. The spores are round. This species differs from the described species in failing to produce fermentation of any of the sugars tried. In dextrose broth a sediment is formed, after some days a very thin incomplete pellicle develops.

Nadsonia fulvescens (fig. 2). This is quite the largest yeast encountered. The vegetative cells are of various shapes, but pear-shaped cells with a small nipple-like projection at one end, similar to those which occur at both ends in *Hansenia apiculata*, are very numerous and characteristic. The protoplasm contains numerous fine highly refractile granules, very rarely a vacuole. The growth in young agar cultures is white and very mucoid in character. As the culture grows older the growth becomes tan and then gradually reddish brown; one strain became almost orange in color. This color change is evidence of spore formation. Guilliermond (11) describes the spores as containing a large fat globule and having a roughened

membrane. The fat globules were found quite regularly, and in some cultures the outer lamella of the thick spore wall peeled off in places giving a very irregular contour. Spores are supposed to be the result of a heterogamic conjugation of a cell (macrogamete) with its bud (microgamete). A considerable number of the sporogenous cells showed an attached bud. Guilliermond (11), reports that this species slowly ferments several sugars, which was not found to be the case with any of these strains. But in morphological characters, in the color of the colonies, and in the characteristic development of white (non-sporogenous) sectors in old colonies it seems identical with *Nadsonia fulvescens*. This yeast was isolated from six different soils.

Torulaspora sp. (fig. 3). The young cells are small and round, without granules and only occasionally showing a vacuole. The sporogenous cells are larger, contain a very prominent large fat vacuole, and in the majority of cases send out spurs which are looked upon as copulation tubes; in no case, however, was evidence of conjugation found. Only one strain was isolated, which gave a dull white growth on Sabouraud agar, and a heavy pellicle on dextrose broth, without fermenting any of the sugars.

Saccharomyces sp. Two species of spore-forming yeasts producing an alcoholic fermentation were isolated. These are designated *A* and *B*.

Saccharomyces A. (fig. 4). The young cells are of medium size and oval. Nearly all contain a large vacuole with a "dancing body," and numerous small granules and vacuoles. Spores were formed rather scantily on plaster blocks, fairly abundantly—two to four—on McKelvey's medium. Agar slant cultures gave a rather restricted growth, moist and shiny, white at first but rapidly becoming brown. Only dextrose was fermented; this yeast therefore belongs in the fourth sub-group of the genus. No pellicle was formed on liquid media. This species was isolated twice.

Saccharomyces B. (fig. 5). The young cells are much larger and rounder than those of the preceding species, resembling *S. cerevisiae*. They also possess very large volutin vacuoles, and numerous small granules. Two to four spores are formed readily on plaster blocks, on McKelvey's medium, and in old agar slant cultures in occasional cells. The growth on Sabouraud agar slant cultures is abundant, moist and white, remaining white for some weeks. In 5 per cent dextrose broth a very vigorous alcoholic fermentation with abundant gas is produced; no pellicle is formed. One strain of this species was found to produce 12 per cent alcohol. Sucrose is also fermented, but none of the other sugars tested is. It therefore belongs in the second sub-group of the genus. It was also isolated twice.

Pichia sp. (fig. 6). The young cells are large, oval to sausage-shaped, and contain large vacuoles. Spores are formed readily in old cultures, on plaster blocks and on McKelvey's medium. They vary from two to four in number, three being found most frequently. The spores are round and each contains a highly refractile fat globule characteristic of the spores of this genus. On Sabouraud agar an abundant spreading dry growth with a dull surface is formed. On dextrose broth a heavy dry scum imprisoning gas bubbles appears. Gas is produced abundantly but rather slowly with most strains. Sucrose is also fermented. This species, isolated from six different soils, could be readily recognized by the strong odor of banana oil (amyl acetate) produced in all cultures with fermentable sugars. This is so striking that the authors believe this species would have been recorded if it had been described previously. Sartory [quoted by Guilliermond (11)] has described a yeast much like *Willia saturna*, isolated from banana leaves, which gave a banana odor.

Willia anomala (fig. 7). This species was readily identified by the hat-shaped spores which were formed on McKelvey's agar and on plaster blocks. The growth on agar was thinner than that of the preceding species; one strain showed a tendency to produce mycelium dipping down into the agar in old cultures, indicating the close relationship to *Endomyces*. On dextrose broth, a thin pellicle was formed and fermentation was rapid and abundant; sucrose and maltose were also fermented. All strains gave the characteristic ethyl acetate odor. This species was found three times.

Torula sp. The majority of the yeasts isolated fell in this genus, i.e., they failed to produce spores, or scums on liquid media, and for the most part gave no fermentation. The descriptions of the many species which have been referred to this genus are particularly incomplete and confusing. One species, *Torula glutinis*, was readily recognized by its characteristic pigment. The remaining varieties have been grouped together as species, but little attempt to identify them has been made because of the hopeless confusion in the literature. In addition to those named, four different species were recognized which have been designated A, B, C, and D.

Torula glutinis (fig. 8). Four strains of yeasts producing a red pigment were isolated from as many different soils. In morphological and cultural characters, these all proved identical with each other and with a strain of *Torula glutinis*, obtained from the American Type Culture Collection, also with a strain isolated from oysters which has been completely described by Hunter (20). In spite of considerable confusion in the literature on red yeasts, it is believed that these strains can be positively identified. The young cells are small and oval, and are relatively free from vacuoles and granules. In old cultures the cells contain a large fat globule. Agar slant cultures are abundant and very mucoid in character. After a time most of the growth slides to the bottom of the tube. In liquid media some turbidity and an incomplete ring pellicle are produced. No fermentation occurs with any of the sugars.

Torula A. (fig. 9). This yeast was found but once. The cells vary in form, an elongated oval being the predominating type. Reproduction appears to be by a process intermediary between budding and fission, as in *Saccharomyces*. The cells contain numerous small, highly refractile fat globules which increase in number with the age of the culture but show no tendency to coalesce. Agar slant cultures are somewhat restricted, smooth and white. In dextrose broth turbidity and sediment are produced without fermentation. None of the other sugars were fermented.

Torula B. (fig. 10). This was obtained from five different soils. The young cells are large and oval, with prominent volutin vacuoles and especially striking "dancing bodies" many of which are of the size and shape of small bacilli. In older cultures the cells are smaller, and more variable both in size and form. Agar slant cultures are smooth and white. In 5 per cent dextrose broth a fairly rapid and abundant fermentation is produced. None of the other sugars is fermented.

Torula C. (fig. 11). The cells are smaller than the preceding, and nearly round. Agar slant cultures are smooth and white. No fermentation occurs with any of the sugars. This yeast was isolated twice.

Torula D. (fig. 12). In this group have been placed sixteen strains isolated from as many different soils, which showed some slight differences, but all of which were characterised by very small cells and practically no fermentation. The illustration is a composite field from young cultures of several different cultures, and shows the degree of variation in morphological characters. In some strains round cells were more numerous, in others elliptical ones, but in no case were these characters sufficiently prominent or constant to warrant making a new group. In old cultures in general the cells were more variable in size and form, and many contained large fat globules. Agar slant cultures are smooth and white. Twelve of the strains gave a very slow and slight fermentation of dextrose, the gas traps being half filled after about a week. Another produced acid from sucrose. Otherwise there was no fermentation.

SUMMARY

Yeasts were found in small numbers in 39 of 87 soil samples. There was no correlation between the occurrence of yeasts and the type of soil, the nature of the crop, or the season of the year. They were found in such small numbers and so haphazard in distribution as to indicate that they play no important part in soil transformations.

Forty-nine strains isolated fell naturally into twelve groups or species. Of these but three, *Nadsonia fulvescens*, *Willia anomala*, and *Torula glutinis*, could be positively identified. The remaining strains were species of *Zygosaccharomyces*, *Torulaspora*, *Saccharomyces* (two species), *Pichia*, and *Torula* (four species).

REFERENCES

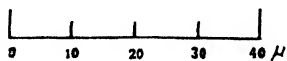
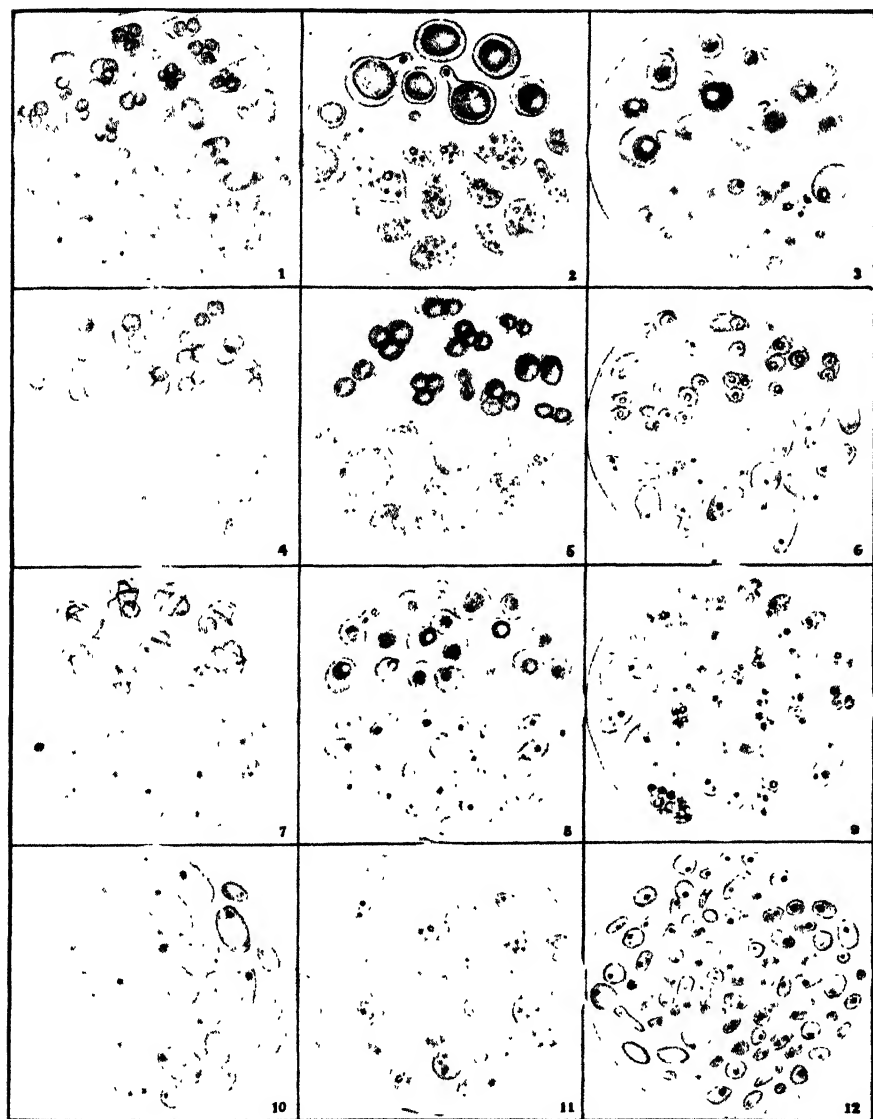
- (1) ADAMENTZ 1886 Untersuchungen über die niedern Pilze der Ackerkrume. Diss. Phil. Leipzig. (Cf. F. Löhnis, Handbuch der landwirtschaftlichen Bakteriologie, p. 516, Berlin, 1910.)
- (2) BERLESE, A. 1897 Über die Verteilung der alkoholischen Fermenten in der Nature. Ueber die Transportmittel der alkoholischen Fermente. *Riv. Patol. Veg.*, v. 5. [Cf. Guilliermond and Tanner (11).]
- (3) BOUTROUX, L. 1881 Sur l'habitat et la conservation des levures spontanees. *Bul. Soc. Linn., Normandie* (3) 5: 120-134.
- (4) BOUTROUX, L. 1883 Les ferments alcooliques. *Bul. Soc. Linn., Normandie* (3) 7: 73-113.
- (5) BOUTROUX, L. 1884 Sur la conservation des ferments alcooliques dans la nature. *Ann. Sci. Nat., Bot.* 17: 144-209.
- (6) BREFELD. 1874-75-76 Botanische Untersuchungen über Gärung. *Landw. Jahrb.* v. 3, 4, 5.
- (7) CORDIER, J. A. 1898 Contribution à la biologie des levures de vin. *Compt. Rend. Acad. Sci. (Paris)* 127: 628-630.
- (8) DEKRUYFF, E. 1908 Untersuchungen über auf Java einheimische Hefearten. *Cent. Bakt. (II)* 21: 616-619.
- (9) FISCHER, H. 1909 Bakteriologische-chemische Untersuchungen. *Landw. Jahrb.* 38: 358.
- (10) FRAZIER, W. C. 1926 A new method for the detection of proteolysis by bacteria. *Jour. Bact.* 11: 80.
- (11) GUILLIERMOND, A., AND TANNER, F. W. 1920 The Yeasts. New York.
- (12) HANSEN, E. C. 1880 Ueber *Saccharomyces apiculatus*. *Hedwigia* 9: 75-77.
- (13) HANSEN, E. C. 1881 Recherches sur la physiologie et la morphologie des ferments alcooliques. *Meddel. Carlsberg Lab.* 3: 159-183.
- (14) HANSEN, E. C. 1882 Recherches sur les organismes qui a differentes epoques de l'annee se trouve dans l'air a Carlsberg et aux alentours et qui peuvent se developper dans la mout de biere. *Compt. Rend. Lab. Carlsberg* 1: 381-454.
- (15) HANSEN, E. C. 1890 Nouvelles recherches sur la circulation du *Saccharomyces apiculatus* dans la Nature. *Ann. Nat., Bot.* 7: 185-192.
- (16) HANSEN, E. C. 1895 Experimental studies on the variation of yeast cells. *Ann. Bot.* 9: 549-560.
- (17) HANSEN, E. C. 1911 Nouvelles recherches sur la circulation des levures dans la nature. *Compt. Rend. Lab. Carlsberg* 9: 39-47. (Also *Cent. Bakt. (II)* 10: 1903.)
- (18) Hansen, E. C. 1911 Sur les foyers des levures alcooliques situés au-dessus de la surface du sol. *Compt. Rend. Lab. Carlsberg* 9: 61-69. (Also *Cent. Bakt. (II)* 14: 545-550.)
- (19) HEINZE, B. 1905 Einige Berichtigungen und weitere Mitteilungen zu der Abhandlung: "Ueber die Bildung und Wiederverarbeitung von Glykogen durch niedere pflanzliche Organismen." Berichtigung bezüglich der Ueberwinterung der Weinhefe. *Cent. Bakt. (II)* 14: 9-21.
- (20) HUNTER, A. C. 1920 A pink yeast causing spoilage in oysters. U. S. Dept. Agr. Bul. 819.

- (21) KLOCKER, A. 1903 Une espèce nouvelle de *Saccharomyces*, *Sacch. saturnis*, Klöcker ayant des spores caractéristiques. *Compt. Rend. Lab. Carlsberg* 6: 84-91.
- (22) KLÖCKER, A. 1906 Die Gärungsorganismen. Stuttgart.
- (23) KLÖCKER, A. 1905-07 Abstammung und Kreislauf der *Saccharomyceten*. F. LAFAR. Handbuch der technischen Mykologie, 4: 148-155. Jena.
- (24) KLÖCKER, A. 1909 Deux nouveaux genres de la famille des *Saccharomycetes*. *Compt. Rend. Lab. Carlsberg* 7: 273-278.
- (25) KLÖCKER, A. 1909 *Endomyces Javanensis*, nov. sp. *Compt. Rend. Lab. Carlsberg* 7: 267-272.
- (26) KOHL, F. G. 1908 Die Hefepilze. Leipzig.
- (27) LUDWIG, R. E. 1918 Etude de quelques levures alpines. *Bul. Soc. Bot. Genève* 9: 431-461.
- (28) MCKELVEY, C. E. 1926 Notes on yeasts in carbonated beverages. *Jour. Bact.* 11: 98-99.
- (29) MÜLLER-THURGAU, H. 1889 Neue Forschungen auf dem Gebiete der Weingärung und deren Bedeutung für die Praxis. *Ber. Verhandl. xl Deut. Weinbaukongress in Trier*, p. 80-100. [Cf. Heinze. *Cent. Bakt.* (II) 11: 355. 1904.]
- (30) MÜLLER-THURGAU, H. 1905 Nachweis von *Saccharomyces ellipsoideus* im Weinbergsboden. *Cent. Bakt.* (II) 14: 296-297.
- (31) PASTEUR, L. 1878 Examin critique d'un écrit posthume de Claude Bernard sur la fermentation alcoolique. *Compt. Rend. Acad. Sci. (Paris)* 87: 813-819.
- (32) PASTEUR, L. 1879 Studies on fermentation. Trans. by F. Faulkner and D. C. Robb. London.
- (33) REUKAUF, E. 1912 Nektarhefen. *Cent. Bakt.* (II) 34: 258-259.
- (34) SCHUSTER, V., AND VLADIMIR, U. 1913 Studien über Nektarorganismen. *Ber. Deut. Bot. Gesell.* p. 129-138. [Cf. *Cent. Bakt.* (II) 42: 96. 1914.]
- (35) VALLERY-RADOT, R. 1923 The Life of Pasteur. Trans. by Mrs. R. L. Devonshire, New York.
- (36) WAKSMAN, S. A. 1922 A method for counting the numbers of fungi in the soil. *Jour. Bact.* 7: 339-341.
- (37) WORTMAN. 1897-98 Cf. Klöcker, A. Die Gärungsorganismen.

PLATE 1. TWELVE SPECIES OF YEASTS FOUND IN SOILS

In figures 1 to 8, inclusive, the upper half shows the morphology in old cultures or cultures from McKelvey's medium for spores; the lower half shows the cells in young Sabouraud agar cultures. In the remaining figures the entire field shows cells from a young culture.

- | | |
|---------------------------------|---------------------------|
| 1. <i>Zygosaccharomyces</i> sp. | 7. <i>Willia anomala</i> |
| 2. <i>Nadsonia fulvescens</i> | 8. <i>Torula glutinis</i> |
| 3. <i>Torulaspora</i> sp. | 9. <i>Torula</i> A. |
| 4. <i>Saccharomyces</i> A. | 10. <i>Torula</i> B. |
| 5. <i>Saccharomyces</i> B. | 11. <i>Torula</i> C. |
| 6. <i>Pichia</i> sp. | 12. <i>Torula</i> D. |



STUDIES ON SOME OBLIGATE THERMOPHILIC BACTERIA FROM SOIL¹

WILLIAM A. FEIRER

Johns Hopkins University

Received for publication April 29, 1926

The investigations of thermophilic bacteria by Barlow (1), Weinzirl (18), Donk (5), and Bigelow and Estey (3), have developed a new interest in this group of organisms because of their significance in the spoilage of canned foods. Thermophilic bacteria have a wide distribution in nature, and their isolation from water, sewage, snow, canned foods, milk, cotton, hay, manure, and soil has been reported by various investigators. As Morrison and Tanner (14) (15) have recently published a careful review of the literature dealing with thermophilic organisms, no historical resumé of this group of bacteria will be presented.

Since two important groups of food products are known to be subject to contamination by thermophilic bacteria, the natural habitat of this group takes on very considerable significance. The literature contains many references to isolations of thermophiles from various sources, but the descriptions of these organisms are so varied and incomplete as to render accurate classification and identification impossible. It is necessary, then, to select the probable habitat of this group and study as many different species as possible, at the same time obtaining a complete and accurate cultural description of each. Only in this manner can the significance and importance of the group be brought into its true position. Nearly all canned foods, as well as milk, are farm products and are intimately related to the soil. It is logical, therefore, to believe that the soil, which is known to contain large numbers of these organisms, is one probable source of the group. The following investigation was undertaken to determine the various types of thermophilic bacteria found in the soil and to obtain an exact cultural description of each of the species isolated.

Twelve samples of the topsoil were collected from fields supporting various types of vegetative growth. Topsoil was used because Globig found that the superficial layer of soil contained the maximum number of bacteria. The topmost two inches was scraped off and a small portion of exposed soil was collected in a test tube containing plain broth. No samples were collected from a depth of more than six inches.

¹ From the Department of Bacteriology of the School of Hygiene and Public Health.

The media for aerobic cultivation were prepared according to standard methods, whereas media usually employed for anaerobic cultivation were made by the methods suggested by Kahn (10). The media were inoculated from the growth on a 24-hour agar slant culture and the reactions were recorded as recommended by the committee on the Descriptive Chart of the Society of American Bacteriologists (13).

All the thermophilic organisms studied grew well at 55°C. and failed to grow at 37°C. when incubated for 28 days. They may therefore be regarded as obligate thermophilic bacteria. The cultures were all bacilli and were easily stained with gentian violet, carbol fuchsin, and methylene blue. Hanging-drop preparations, for the observation of motility, were made from peptone water cultures, incubated at 55°C. for 12 hours. Motility of the organisms was more easily observed with the aid of the dark-field microscope. Without exception, the cultures isolated were motile. For staining flagella, Löffler's and Plimmer and Paine's methods (16) were employed repeatedly but without any definite results.

The usual anaerobic media were employed in this investigation to observe proteolytic action and certain peculiar cultural characteristics. Cooked meat, brain, alkaline egg, and egg cube media were inoculated and incubated for 28 days at 55°C.

McLeod and Gordon (12) have classified bacteria according to their sensitivity to hydrogen peroxide and their production of catalase. The production of catalase by the cultures described in this paper was determined by scraping a platinum loop over the surface of a 24-hour culture and immersing it in a dilute solution of hydrogen peroxide. A rapid evolution of gas indicates the presence of catalase. McLeod and Gordon found that the great majority of aerobic bacteria produce catalase and it is of interest to note that all the aerobic thermophiles described in this study give this reaction.

The cultures were not pathogenic for guinea pigs when injected subcutaneously, intramuscularly, or intraperitoneally, or when fed by mouth.

THERMAL DEATH POINT DETERMINATION

For the determination of thermal death points in this investigation the method set forth by Bigelow and Estey (3) was followed with a few modifications. Nutrient broth tubes were inoculated with pure cultures of the organism to be tested and incubated at 55°C. for 48 hours to insure good growth. The actively growing broth cultures were transferred to flasks containing 100 cc. of nutrient broth and incubated for 7 days at 55°C. and then for 2 days at 65°C. At the end of this incubation period the flasks were heated to 90°C. for 30 minutes to kill any vegetative forms present, cooled immediately, and stored in the refrigerator to prevent the germination of spores. These broth cultures were centrifugalized and washed three times in sterile salt solution and again transferred to sterile flasks. The concentration of the washed spores was determined by plating out dilutions, and the stock

solutions were diluted, when necessary, to bring the concentration to 2,500,000 spores per cubic centimeter of suspension.

The tubes used for the thermal death point determination were of hard glass, averaging 153 mm. in length, 11 mm. inside diameter, and the wall 0.9 mm. in thickness. They were soaked overnight in 0.1 N hydrochloric acid, rinsed thoroughly with distilled water, and sterilized in the autoclave with 4 cc. of normal salt solution in each tube. These tubes were inoculated with 1 cc. of the stock suspension of spores, sealed off to an average of 135 mm., and held at a low temperature until ready to be heated.

A De Khotinsky electric bath containing "Crisco" was used to maintain a constant temperature. Before immersing the tubes in the oil bath, the temperature was raised two degrees above the temperature used for the test, to compensate for the loss due to the introduction of the tubes, and two minutes were allowed for the heat to reach the center of the tubes and for the temperature of the bath to become constant. When a temperature above 130°C. was used, it was found necessary to immerse the tubes in a boiling water bath for one minute and allow for a 3-degree fall in the temperature when the tubes were introduced into the oil bath.

After the initial change in temperature caused by the tubes was allowed for, the oil bath was found to remain constant over the period of heating. No greater rise or fall than 0.5° was observed.

The thermal death point of the spores was determined as follows:

The tubes containing the suspension of spores were held at the desired temperature for 15 minutes and were then removed and placed in the refrigerator to cool rapidly. When cool, the salt suspensions were transferred to sterile broth tubes and incubated for 48 hours. The initial temperature used was 100°C. and tests were made at every increasing 5° up to 160°C. Sterility tests were made by inoculating agar slants with the broth cultures and examining for growth after 24 hours' incubation. Tubes which were sterile were further incubated for 7 days, but it was found that 48 hours' incubation was sufficient time for the vegetation of any viable spores that might have survived the heating. Throughout the thermal death point determination the media used were all carefully adjusted to neutral with brom cresol purple.

SPECIES ISOLATED

Thermobacillus diastasius, nov. sp.

Two cultures of the *Thermobacillus diastasius*, nov. sp. type were isolated. They are large, spore-forming rods with rounded ends and have a granular protoplasm when stained. The average dimensions of the rods are 6.0 by 1.0 μ . The vegetative cells retain the gentian violet in the Gram stain. Oval spores are formed at the end of the bacillus and measure from 1.0 to 1.25 μ in diameter. The cultures are actively motile.

Acid fermentation but no gas was observed in dextrose, maltose, saccharose, salicin, and glycerin broth. These cultures failed to ferment lactose, starch, inulin, mannitol, or dulcitol. The tests for hydrogen sulfide and indol were negative, and nitrates were not reduced. No change was produced in meat, egg cube, brain, or alkaline egg. Löffler's blood serum was liquefied after 3 days' incubation and gelatin after 4 days'. Litmus milk was coagulated after 48 hours and the litmus decolorized with the exception of an acid zone, about 1 cm.

thick, in the upper portion of the medium. The growth on potato was abundant and spreading.

Thermobacillus diastasius was not pathogenic for guinea pigs.

The thermal death point of the spores was between 130° and 135°C. when exposed for 15 minutes.

Remarks. This bacillus is a true aerobic thermophile isolated from the soil. With the exception of the reduction of nitrates to nitrites, this organism corresponds to *Bacillus thermodiastaticus*, reported by Bergey. The description of Bergey's organism is, however, not complete enough to identify this culture, since he does not include carbohydrate reactions, which are distinctive cultural features of this species.

Thermobacillus vulgaris, nov. sp.

Two cultures of the *Thermobacillus vulgaris*, nov. sp. type were isolated. The organism is rod-shaped with rounded ends and granular protoplasm, and is actively motile. The bacillus averages 3.0 by 0.48 μ . The vegetative cells are Gram-negative and form long chains composed of from 2 to 10 members. Spores are readily formed at the end of the rod.

On plain agar slants the growth of this organism was moderate or slight, spreading, flat, and smooth. In nutrient broth there was a slight haze or cloudiness, but no pellicle formation or sediment.

This culture did not ferment dextrose, maltose, lactose, saccharose, salicin, starch, inulin, glycerin, mannit, or dulcite. Nitrates were not reduced, nor was hydrogen sulfide or indol formed. No change in meat, brain, egg cube, alkaline egg, blood serum, or milk was noted. Gelatin was liquefied only after 28 days' incubation.

Cultures of this organism were not pathogenic for guinea pigs.

The thermal death point for this organism is between 120° and 125°C. for 15 minutes.

Remarks. Morphologically this organism is similar to *Bacillus nondiasticus* described by Bergey. The organism reported by Bergey does not liquefy gelatin but reduces nitrates to nitrites and produces a slight acidity in milk. *Thermobacillus vulgaris* liquefies gelatin, does not reduce nitrates or alter litmus milk medium.

Thermobacillus digestans, nov. sp.

Four cultures of *Thermobacillus digestans*, nov. sp. type were isolated. This bacillus has rounded ends and granular protoplasm, with average dimensions of 5.6 by 0.8 μ . The vegetative cell is Gram-negative and often forms short chains. The spores are oval and are located sub-terminally in the rod. Active motility.

An abundant, spreading, flat, glistening, and smooth growth was observed on plain agar slants. In nutrient broth a heavy pellicle was formed which settled to the bottom after 48 hours.

This organism fermented dextrose, maltose, and mannit with the production of acid but no gas. There were no changes in lactose, saccharose, salicin, starch, inulin, glycerin, or dulcite. Nitrates were reduced to nitrites in 24 hours and there was an evolution of free nitrogen gas. No hydrogen sulfide or indol was produced. In cooked meat medium this organism produced a slow but definite digestion, reducing the volume of meat particles by one half after 28 days' incubation, but without a foul odor. No amino acid crystals were formed after 28 days' incubation. In egg cube medium the albumen showed translucent areas after 2 days' incubation and was completely translucent after 7 days. This cannot be regarded as a true digestion, however, as the cube retained its original size and the edges were well defined throughout the entire period of observation.

In brain and alkaline egg no alteration in the medium was observed. Blood serum was partially liquefied, and after 48 hours a tube containing 30 cc. of gelatin was completely liquefied. Milk was coagulated with complete digestion after 7 days.

This culture produced no pathological changes when injected into a guinea pig.

The thermal death point lies between 145° and 150°C. for 15 minutes.

Remarks. No organism described by other investigators appears to have cultural reactions similar to those of this thermophile. MacFadyen and Blaxall (11) and Dupont (6) report the isolation of thermophilic bacteria which were proteolytic in character, but give no further description. *Thermobacillus digestans* is a true aerobic thermophile, which exhibits proteolytic activity.

Thermobacillus reductans, nov. sp.

Two cultures of the *Thermobacillus reductans*, nov. sp. type were isolated. This bacillus has rounded ends and a granular protoplasm, measures 3.0 μ in length by 0.6 μ in width, and is Gram-positive and actively motile. The spores are terminal or subterminal and are oval.

The growth on plain agar slants was abundant, spreading, glistening, flat, and smooth. Nutrient broth cultures were cloudy, but there was no pellicle formation.

Dextrose, maltose, saccharose, salicin, and starch were fermented, with the production of acid but no gas. No change in the H-ion concentration was observed in lactose, inulin, glycerin, mannitol, or dulcitol. Nitrates were reduced to nitrites, but no hydrogen sulfide or indol was formed. There was no alteration in meat, egg cube, brain, or alkaline egg medium. Gelatin and Löffler's blood serum were slowly liquefied. Litmus milk was acidified and coagulated with a slowly progressing peptonization until the medium became translucent.

This organism was not pathogenic for guinea pigs.

The thermal death point for this culture lies between 135° and 140°C. at 15 minutes' exposure.

Remarks. *Thermobacillus reductans* is an aerobic thermophilic organism isolated from soil. In some few respects it corresponds to *Bacillus thermoindifferens*, described by Weinzierl (18). However, the organism described by Weinzierl does not reduce nitrates and grows equally well between 20° and 55°C. The culture described above reduces nitrates to nitrites and has a minimum temperature of growth at 40°C.

Thermobacillus restatus, nov. sp.

Three cultures of the *Thermobacillus restatus*, nov. sp. type, having the same morphology and exhibiting identical reactions, were isolated. This sporeforming bacillus averages 1.6 by 0.8 μ in size and is Gram-positive and actively motile. The spores, when present, are oval and usually situated subterminally.

On plain agar slants the growth was abundant, spreading, flat, glistening, and smooth. No scum was formed in broth, but a veil-like sediment was found at the bottom of the tube.

There was no gas produced by this culture in carbohydrate media, but dextrose and maltose were fermented with the production of acid. Nitrates were not reduced and this culture did not break down peptone to produce indol. On lead acetate agar this organism showed a black growth, which indicated the production of hydrogen sulfide. In meat, egg cube, brain, alkaline egg, blood serum, gelatin, and milk media no change was observed.

This organism was not pathogenic for guinea pigs.

At 15 minutes' exposure the thermal death point of this organism lies between 150° and 155°C.

Remarks. Blau (4) isolated a thermophile, *Bacillus robustus*, which corresponds in some respects to *Thermobacillus restatus*. He fails, however, to observe the action on nitrate and several other media, and does not record the production of hydrogen sulfide. It is therefore impossible to make an exact comparison of *Bacillus robustus* and *Thermobacillus restatus* to establish their identity.

Thermobacillus ruber, nov. sp.

Thermobacillus ruber, nov. sp. has rounded ends and granular protoplasm, is Gram-positive, and the spores are oval and are situated at the end of the rod. It is actively motile and the average dimensions are 4.0 by 0.6 μ .

In plain nutrient media this organism grew well. Acid but no gas was formed in dextrose, maltose, saccharose, salicin, starch, glycerin, and mannit. Lactose, inulin, and dulcitol were not fermented. Nitrates were not reduced and there was no production of hydrogen sulfide or indol. In cooked meat and brain a deep pink color was produced but no further change. Blood serum was not liquefied, but a pink pigment was produced by the growth of this organism. No change in egg cube, alkaline egg, gelatin, or milk medium was noticed.

This organism was not pathogenic for guinea pigs.

The thermal death point for this organism for 15 minutes lies between 135° and 140°C.

Remarks. So far as the writer is aware, there is no thermophile described in the literature which is similar to this organism. It is true, however, that former workers did not employ the media on which *Thermobacillus ruber* produces its characteristic pink pigment. On plain agar or infusion agar this culture did not elaborate any coloration, but in meat, brain, and blood serum a definite pink pigment was formed within 48 hours.

Thermobacillus alcalinus, nov. sp.

Four cultures of the *Thermobacillus alcalinus*, nov. sp. type were isolated. The bacillus was Gram-negative, motile, with an average dimension of 6.4 by 0.8 μ . The spores are round, located terminally and frequently subterminally.

The growth on plain agar slants was abundant, spreading, flat, glistening, and smooth. Nutrient broth was cloudy but no pellicle was produced.

Acid without gas was formed in dextrose, maltose, saccharose, salicin, starch, and mannit. Lactose, inulin, glycerin, and dulcitol were not fermented. Nitrates were not reduced, gelatin and blood serum were not liquefied, and there was no production of hydrogen sulfide or indol. No change in meat, egg cube, brain, alkaline egg, or milk was observed.

This organism was not pathogenic for guinea pigs.

The thermal death point for a definite concentration of spores of this culture, exposed for 15 minutes, lies between 125° and 130°C.

Remarks. *Thermobacillus alcalinus* is a true aerobic thermophile isolated from soil. Weinzierl described an organism, which he called *Bacillus aerothermophilus*, that was in some respects similar to this organism. Weinzierl's organism did not reduce nitrates and fermented dextrose, sucrose, and mannitol, but litmus milk was reduced, while *Thermobacillus alcalinus* produced no change in this medium.

Thermobacillus violaceus, nov. sp.

Four cultures of the *Thermobacillus violaceus*, nov. sp. type were isolated. They were all Gram-negative, motile, spore-forming bacilli, averaging 3.0 by 0.8 μ . The growth on agar slants was spreading, flat, glistening, and smooth. Growth in nutrient broth was characterized by cloudiness, present at the end of 12 hours.

Acid was formed in dextrose and saccharose media, but no gas was evolved. No fermentative action was detected on maltose, lactose, salicin, starch, inulin, glycerin, mannit, or dulcitol. Nitrates were not reduced to nitrites and this culture produced no hydrogen sulfide or indol. Meat, egg cube, brain, and alkaline egg media were not altered by this culture. Gelatin was liquefied after 48 hours and blood serum showed a slight liquefaction after 7 days' incubation. Litmus milk was not coagulated, but the medium became deep purple, which was distinctly different from that of the control tube.

This organism was not pathogenic for guinea pigs.

The thermal death point of this culture was between 135° and 140°C. when exposed for 15 minutes.

Remarks. *Thermobacillus violaceus* is an aerobic thermophilic organism isolated from soil. In some few respects it corresponds to *Bacillus thermoalimentophilus*, described by Weinzirl. However, the organism isolated by Weinzirl reduced nitrates to nitrites and produced no acid in carbohydrate media.

Thermobacillus linearis, nov. sp.

Five cultures of the *Thermobacillus linearis*, nov. sp. type were isolated. The bacilli are actively motile, Gram-positive, appear singly in the smears, and average 4.0 by 0.8 μ . Spores are round, usually terminal, and slightly greater in diameter than the sporangium, causing a bulging of the rod.

On plain agar slants the growth was heavy, spreading, glistening, opaque, and smooth. Broth cultures were cloudy, with a slightly mucoid sediment, but no pellicle was observed.

Acid was formed from dextrose, maltose, saccharose, salicin, inulin, glycerin, and mannitol, while lactose, starch, and dulcitol were not fermented. Nitrates were not reduced and there was no production of hydrogen sulfide or indol. No definite alteration of meat, egg cube, brain, or alkaline egg media was produced. Blood serum and gelatin were not liquefied and no change in milk was observed.

An actively growing broth culture of this organism was not pathogenic for guinea pigs.

The thermal death point for a definite concentration of spores for 15 minutes' exposure lies between 135° and 140°C.

Remarks. This organism is in some respects similar to *Bacillus thermotranslucens*, described by Bergey (2). The description of Bergey's organism is, however, not complete enough to identify this culture, since he does not include carbohydrate reactions, which are distinctive features of this species.

Thermobacillus catenatus, nov. sp.

The two cultures of *Thermobacillus catenatus*, nov. sp. that were isolated are bacilli with rounded ends and granular protoplasm. The vegetative cells are Gram-negative, motile, and measure 3.0 μ in length by 0.8 in diameter. Spores, when present, are oval and terminally located.

On plain agar slants the growth of this organism was abundant, definitely spreading, flat, glistening, opaque, and smooth. Broth cultures were cloudy, with a thin, semitransparent film after 24 hours. Neither acid nor gas was formed from any of the carbohydrates used. Nitrates were not reduced. Hydrogen sulfide was produced, as indicated by a black growth on lead acetate agar. Indol was produced by this culture, but was only demonstrated by distilling off the indol, as suggested by Goré. Meat, egg cube, brain, and alkaline egg were not altered by this culture. Löffler's blood serum, after 4 days, was slowly liquefied, and gelatin, after 48 hours. Litmus milk was first coagulated and then slowly peptonized.

This culture was not pathogenic for guinea pigs.

The thermal death point of this culture lies between 135° and 140°C. when exposed for 15 minutes.

Remarks. This organism cannot be correlated with any description of thermophilic bacteria reported in the literature. Morrison and Tanner (14) reported the isolation of 52 cultures of thermophiles from water, all of which produced indol, but their descriptions are not complete enough to identify this culture.

DISCUSSION

The motility of this group of organisms presents some interesting considerations. It is generally believed that bacilli are motile through the lashing action of delicate, whiplike appendages called flagella. The sinuous or worm-like motility seen among the spirochaetes has never been observed in cultures of bacilli, and the active movement of a rod-shaped organism has come to be regarded as evidence of the presence of flagella. These delicate organs of locomotion do not take the ordinary dyes, and a variety of special staining methods have been devised for their demonstration. The technique employed is very exact, since the flagella are easily broken off from the cells and become lost in the surrounding medium. The methods of Löffler and Plimmer and Paine (16) were employed in attempts to stain any flagella present on the thermophiles studied in this investigation. Careful control of the technique was obtained by staining the peritrichic flagella on *Bacillus coli*. Both methods were equally successful, as the flagella of *Bacillus coli* were plainly seen under the microscope. When applied to the thermophiles under the same conditions, however, both methods failed to demonstrate flagella, either attached to the bacilli or broken off and free on the microscope slide. There may be some difference in the chemical composition of the flagella of thermophiles, because of their adaptation to growth at high temperatures, which makes the usual staining methods valueless. There is the possibility, however, that the motility of these organisms is not due to flagella but to some peculiar contractile property of the cytoplasm. Although the bacilli appeared rigid when examined in living preparations under the dark-field microscope, the internal arrangement of the cells could not be seen. When bacilli with flagella are examined under the dark-field, frequent peculiar flashes of light are caused by the rapid vibration of the flagella. This phenomenon was never observed in the examination of any of the thermophiles studied. The cause of motility in these cultures must, therefore, remain undetermined for the present.

MacFadyen and Blaxall (11) isolated from various sources several thermophilic bacteria, which grew at a temperature between 60° and 65°C.; their most marked property was the decomposition of protein bodies. Dupont isolated from manure a thermophilic bacterium which he described as being proteolytic in character. In this investigation *Thermobacillus digestans* shows a slow but definite proteolytic action. Gelatin and blood serum are liquefied, cooked meat medium is digested but no putrefactive odor is detected, and coagulated egg albumen is made translucent by the action of this organism.

Rettger (17), in his investigation on putrefaction, concludes that it takes place only under strictly anaerobic conditions, that it is always accompanied by the production of a foul odor, and that its end products are substances such as mercaptans. For example, *Bacillus sporogenes*, an obligate anaerobe,

when grown in cooked meat medium, alkaline egg, or egg cube medium, causes the dissolution of these substances, giving rise to foul-smelling gases. In contrast to this reaction, the apparent decomposition of the medium by aerobic bacteria is never accompanied by foul odors nor a reduction in the bulk of the protein substances included in the medium.

On the other hand, other investigators maintain that liquefaction of gelatin, blood serum, or of coagulated milk is an indication of proteolytic activity.

The action of *Thermobacillus digestans* on egg cube medium is also of particular interest. When incubated in this medium for 7 days at 55°C. the tube of coagulated albumen became perfectly translucent. This type of reaction suggested the possible presence of an extracellular enzyme liberated by the bacilli. Filtration experiments already described were negative, however, since the sterile filtrate of an actively growing culture in egg cube medium failed to produce translucency of the egg cube in the complete absence of the bacteria. It seems evident that this reaction takes place only when the culture is growing in the medium. A similar reaction on this medium is produced by certain anaerobes. Thus *Bacillus botulinus*, after 3 days' incubation at 37°C., produces gas in egg cube medium, but the cube is two-thirds digested, whereas the remaining portion is rather translucent. Likewise the anaerobes *Bacillus putrificus* and *Bacillus bifermentans*, as described by Kahn (10), produce a translucency of the egg cube, but there is also a digestion, as indicated by the fact that the edge becomes scalloped. *Thermophilus digestans* produces a complete translucency of the egg cube, but even after 28 days' incubation at 55°C. the edges are well defined and there is no indication of digestion.

CONCLUSIONS

1. The biological and cultural activities of ten aerobic, spore-forming, motile, obligate thermophiles are described.
2. The optimum temperature for growth falls between 50° and 60°C. Growth does not take place below 40°C. and ceases when the temperature is raised to 80°C.
3. The thermal death point for a definite concentration of spores varies with each species. The limits lie between 125° and 155°C. for 15 minutes.
4. One aerobic obligate thermophile is described which exhibits proteolytic action.
5. The cultures are not pathogenic for guinea pigs.

• REFERENCES

- (1) BARLOW, B. 1912 A spoilage of canned corn due to a thermophilic bacterium. Thesis for Degree of Master of Science. Univ. Ill.
- (2) BERGEY, D. 1919 Thermophilic bacteria. *Jour. Bact.* 4: 301.
- (3) BIGELOW, W. D., AND ESTEY, J. R. 1920 The thermal death point in relation to time of typical thermophilic organisms. *Jour. Infect. Diseases* 27: 602.

- (4) BLAU, O. 1906 Über die temperatur maxima der Sporeukermung. *Centbl. Bakt.* (II) 15: 97.
- (5) DONK, P. J. 1920 A highly resistant thermophilic organism. *Jour. Bact.* 5: 373.
- (6) DUPONT, C. 1902 Sur les fermentations aerobies du furnier de ferme. (Original not seen. See *Jour. Bact.* 7: 350. 1922.)
- (7) GEORGEVITCH, P. 1910 Bacillus thermophilus Losanitchi, nov. spec. *Centbl. Bakt.* (II) 27: 150.
- (8) GLOBIG. 1888 Über Bakterienwackstum bei 50-70°C. *Ztschr. Hyg. u. Infektionskrank.* 3: 294.
- (9) GORÉ, S. H. 1921 The cotton-wool plug test for indol. *Indian Jour. Med. Research.* 8: 505.
- (10) KAHN, M. C. 1922 A cultural study of anaerobic sporebearing bacteria. *Jour. Med. Res.* 43: 155.
- (11) MACFADYEN, A., AND BLAXALL, F. R. 1896 Thermophilic bacteria. *Jour. Path. and Bact.* 3: 87.
- (12) McLEOD, J. W., AND GORDON, J. 1923 Catalase production and sensitiveness to hydrogen peroxide amongst bacteria. *Jour. Path. and Bact.* 26: 326.
- (13) Manual of Methods for Pure Culture Study of Bacteria, 1923, Soc. Amer. Bact.
- (14) MORRISON, L. E., AND TANNER, F. W. 1922 Studies on thermophilic bacteria. *Jour. Bact.* 7: 343.
- (15) MORRISON, L. E., AND TANNER, F. W. 1924 Studies on thermophilic bacteria. *Bot. Gaz.* 77: 171.
- (16) PLIMMER, H. G., AND PAINE, S. G. 1921 A new method for staining bacterial flagella. *Jour. Path. and Bact.* 24: 286.
- (17) RETTGER, L. F. 1908 Studies on putrefaction. *Jour. Biol. Chem.* 4: 45.
- (18) WEINZIRL, J. 1918 The bacteriology of canned foods. *Jour. Med. Res.* 39: 349.

MINNESOTA GLACIAL SOIL STUDIES: II. THE FOREST FLOOR ON THE LATE WISCONSIN DRIFT¹

FREDERICK J. ALWAY AND PAUL M. HARMER²

University of Minnesota

Received for publication April 22, 1926

INTRODUCTION

In studies of the soils of American forests, little attention usually has been devoted to the forest floor, or to the part of this known as leafmold, as distinct from the immediately underlying five or six inches of soil. This is because most of such soil studies have been made from a strictly agricultural viewpoint and not from that of either forestry or soil science. Where the timber has been cut off, and the land used as a pasture for some time before removing the stumps, the layer of leafmold has usually become thin and firmly bound by the grass roots to the mineral matter below. When the sod is plowed, whether or not this has been preceded by the removal of all the stumps, the leafmold is turned under and whatever plant nutrients and soil ameliorating materials it carries become integral parts of the surface soil. Even if the original forest trees still remain at the time of sampling, or if there should be a second growth of trees and brush, a great change in the forest floor may take place before the land is plowed.

The term *forest floor* is here used "to designate only the deposits of vegetable matter on the ground in a forest" as proposed in 1917 by a committee on forest terminology of the Society of American Foresters and of the Canadian Society of Forest Engineers (2, p. 78) which further proposed three subdivisions of the forest floor:

1. *Litter*—the upper, but slightly decomposed, portion of the forest floor;
2. *Leafmold*, or *humus*—the portion in which decomposition of the litter is so far advanced that its original form is not distinguishable (2, p. 82);
3. *Duff*—an intermediate layer of more or less decomposed organic matter between the litter and the leafmold.

The term *humus*, already so variously used by soils investigators, does not appear to the authors as satisfactory as the already well established synonym—*leafmold* (2, p. 85).

¹ Published with the approval of the Director as Paper No. 614 of the Journal Series of the Minnesota Agricultural Experiment Station.

² Formerly Research Assistant, Division of Soils, Minnesota Agricultural Experiment Station; now Research Associate at Michigan State College of Agriculture and Applied Science.

The data presented in this paper were secured in a study of virgin forest soils compared with virgin prairie soils of the same age, both developed on the same calcareous glacial drift—the till plains of the Late Wisconsin glaciation. In this, the authors took extreme precautions, both to select for sampling only those woods which were most nearly virgin and to separate sharply the leafmold layer from the underlying mineral soil, the first foot of which was sampled in three-inch sections.

With the more or less disintegrated plant debris were included whatever fallen leaves and small woody fragments were present, but all living plants that might be within the selected squares were rejected. Thus the term *forest floor* is used in the narrow sense of "Waldstreu," as defined by Ramann



FIG. 1. MAP OF MINNESOTA SHOWING LOCATIONS OF WOODS SAMPLED

(5, p. 462), and not in its wider sense, which would include the soil cover and accordingly the low-growing plants which occur under the protection of the forest.

The samples were collected in August and September, 1916, from nine wooded fields; three near Hamel, three near Taylors Falls, and three near Mizpah (table 1). The fields of the first group lie about 20 miles west of St. Paul and remain from the deciduous forest known as the Big Woods of Minnesota. Those of the second group are about 40 miles northeast of St. Paul, lying at the eastern edge of the state, and form remnants of a forest very similar to the Big Woods. The Mizpah group is about 200 miles north of St. Paul and well within the coniferous forest (fig. 1).

TABLE 1
Location of woods sampled

GROUP	FIELD NUMBER	RANGE, WEST	TOWNSHIP, NORTH	SECTION	PART OF SECTION
Hamel.....	I	23	118	2	SE $\frac{1}{4}$ of NE $\frac{1}{4}$
	II	23	119	16	NE $\frac{1}{4}$ of SE $\frac{1}{4}$
	III	24	119	25	SE $\frac{1}{4}$ of NW $\frac{1}{4}$
Taylors Falls	I	19	34	22	SW $\frac{1}{4}$ of SE $\frac{1}{4}$
	II	19	34	10	SW $\frac{1}{4}$ of SW $\frac{1}{4}$
	III	19	35	31	NW $\frac{1}{4}$ of NW $\frac{1}{4}$
Mizpah.....	I	27	152	30	NE $\frac{1}{4}$
	II	27	152	10	NE $\frac{1}{4}$
	III	28	151	10	E $\frac{1}{2}$

TABLE 2
Climatological data

GROUP	APPROXIMATE ELEVATION	LENGTH OF RECORD	LENGTH OF GROWING SEASON	MEAN TEMPERATURE					SNOW FALL	PRECIPITATION
				Winter	Spring	Summer	Fall	Year		
	feet	years	days	°F.	°F.	°F.	°F.	°F.	inches	inches
Hamel*.....	1,000	34	150	16	44	70	48	45	44	28.39
Taylors Falls 	950§	34	140	12	42	67	45	42	52	31.46
Mizpah†.....	1,400	38	100	8	38	65	42	38	50	25.33

* Record from Minneapolis, 13 miles distant.

† Record from Grantsburg, Wis., 30 miles distant.

‡ Record from Winnibigoshish, 35 miles distant.

§ Approximate elevation of woods and not of the U. S. Weather Bureau station, which is in the St. Croix Valley and only 759 feet above sea level.

At Hamel and Taylors Falls the climate is much alike, whereas at Mizpah both the temperature and rainfall are lower, the growing season is much shorter and the duration of the snow cover is much longer (table 2).

CHARACTER OF FOREST GROWTH

The woods at Hamel and Taylors Falls either belong to the maple-basswood type of forest or form a transition from this to the oak-maple type, whereas those at Mizpah belong to the spruce, balsam-birch type (4).

Hamel group

Of the Hamel group, I was the most nearly virgin, only a few trees having been removed from it. Hard maples (*Acer saccharum*) were the most nu-

merous trees, with some red oak (*Quercus rubra*), bur oak (*Quercus macrocarpa*), and basswood (*Tilia americana*) and a few elm (*Ulmus americana*) and ash. Considerable undergrowth of young maples and a few ferns were present but there was no grass. Field II, five miles northwest of the preceding, showed more alteration; scattered tufts of grass were present but these were avoided in sampling. Hard maples and elms were most numerous, with some basswood, red oak, and a very few butternut trees (*Juglans cinerea*). There was about as much undergrowth as in I, consisting of young maples and elms with a few ferns. From III, which lay to the west of the others and about 5 miles from each, still more trees had been removed. Although somewhat more grass was present, the most of the forest floor showed none. Samples were taken only from grass-free sites. About 90 per cent of the trees were hard maples, with elms next in number. The general character of the interior of the Hamel woods may be seen from plate 1, showing II and III.

Taylors Falls group

The most nearly virgin wood in the Taylors Falls group was I, in which basswoods predominated, with many butternuts and smaller numbers of bur oak, white birch (*Betula papyrifera*), and poplar (*Populus tremuloides*). Many small elms were present and ferns were numerous. Wood III appeared almost as nearly virgin as I; very little grass was observed and only three large stumps were found in the six acres. About 80 per cent of the trees were hard maples and the remainder basswood, except for a few elms and butternuts and a very few large white oaks (*Quercus alba*). Ferns were abundant (plate 1). More grass and large stumps were found in II than in the others. In it also the trees were mostly hard maples, with a considerable number of basswood and a few white birch; the undergrowth included ferns and many young elms.

Mizpah group

In the Mizpah group the conifers predominated. Field I was occupied by balsam (*Abies balsamea*) and spruce, with a few white birch; no trees had been cut. From II, in which white cedars (*Thuja occidentalis*) were most numerous along with fewer balsam and some white birch, some of the larger cedars had been removed. In III the trees were chiefly balsam, many of them small, with a few spruce, white birch, white cedar, and balsam poplar (*Populus balsamifera*). The forest floor was thicker in this wood than in any of the eight others.

METHODS OF SAMPLING AND ANALYSIS

As criteria of the degree of virginity of the forest, in selecting the woods for sampling, the forest floor, the amount of grass, the presence of undergrowth, evidences of forest fires in the past, and the extent to which trees had been

removed, as indicated by the stumps, were considered. After a wood had been definitely decided upon, a level uniform area within it was chosen for the actual sampling.

In order to determine the weight of the forest floor per unit area and to secure samples for analysis, a square wooden form similar to an ordinary picture frame, with inside dimensions of exactly 12 by 12 inches was used. This was placed on the ground and any living plants within it were cut off and thrown away. Then with a sharp knife, a square foot section of the floor was carefully separated from the underlying mineral soil and placed in a sack. From 5 to 10 such samples were taken in each wood, the exact number depending upon the uniformity in thickness of the forest floor. The composite sample was well mixed and weighed, and a weighed portion of it saved for a moisture determination at 100°C. The rest was later dried and thoroughly ground before being used in the laboratory studies.

In the case of each sample the authors determined the moisture equivalent at 1000 times gravity, nitrogen by the Kjeldahl-Gunning method, volatile matter by prolonged ignition at dull redness and acidity by the Truog method. For the organic carbon and phosphoric acid, composites prepared from equal weights of the samples from the three woods in each group were used. The organic carbon was determined by mixing the dried material with copper oxide and burning in a current of oxygen, and the phosphoric acid by igniting, extracting the resulting ash with aqua regia and using this extract for precipitation.

For comparison with the forest floor, tables 3, 4, and 5 show some data on the underlying 6 inches of soil. In all the woods this was sampled in two sections 1 to 3 and 4 to 6 inches. In the Hamel and Taylors Falls groups the density of each of the two sections was determined (3). In arriving at the values for the 6 inches, such data as the amount of nitrogen and of volatile matter in each of the two sections have been computed separately and the sum of the two used instead of multiplying the average weight per acre—6 inches by the average percentage of nitrogen or of volatile matter. This is necessary in order to secure correct values, because the first 3-inch section is much less dense than the second while much richer in nitrogen and organic matter.

In the computations of the data for Mizpah woods, in which no density determinations were made, the averages of the densities found for the first and second sections in the Hamel and Taylors Falls woods were used.

AMOUNT AND CHARACTER OF FOREST FLOOR

Amount

The quantity per unit area varies greatly from wood to wood, at Hamel from 0.58 to 1.28 pounds of oven-dry material per square foot, at Taylors Falls from 0.90 to 0.96, and at Mizpah from 1.07 to 4.44. The first is equiva-

TABLE 3
Amount, properties, and composition of forest floor compared with underlying 6 inches of soil

GROUP	FOREST FLOOR				SURFACE 6 INCHES OF SOIL			
	Wood I	Wood II	Wood III	Average	Wood I	Wood II	Wood III	Average
<i>Weight per square foot of dry material, in pounds</i>								
Hamel.....	1.15	1.28	0.58	1.00	31.3	36.2	34.6	34.0
Taylor's Falls ..	0.93	0.96	0.90	0.93	32.3	32.4	34.9	33.2
Mizpah.....	1.39	1.07	4.44	2.30
<i>Color, when moistened to the moisture equivalent</i>								
Hamel.....	Dark grayish brown	Brown	Grayish brown	Grayish black	Dark gray	Dark gray
Taylor's Falls ..	Reddish brown	Light brown	Brown	Dark gray	Dark gray	Dark gray
Mizpah	Reddish brown	Reddish brown	Dark reddish brown	...	Gray	Very light gray	Dark gray
<i>Acidity, determined by Truog method</i>								
Hamel.....	None	None	None	Slight	Slight	Slight
Taylor's Falls ..	None	None	None	Slight	Slight	Slight
Mizpah.....	Slight	Slight	Slight	...	Very slight	Slight	None

Volatile matter, in per cent

Hamel.....	51.8	57.3	62.6	57.2	5.99
Taylor's Falls.....	62.2	63.8	62.3	62.8	5.37
Mizpah.....	80.7	76.4	61.6	72.9	2.77

Moisture equivalent

Hamel.....	97.9	111.1	97.0	102.0	26.0	24.5	20.7	23.7
Taylor's Falls.....	112.8	111.4	112.6	112.3	19.4	20.8	21.2	20.5
Mizpah.....	119.4	120.2	108.8	116.1	17.7	13.8	17.4	16.3

Nitrogen, in per cent

Hamel.....	1.53	1.57	1.76	1.62	0.232	0.190	0.173	0.198
Taylor's Falls.....	1.86	1.74	1.82	1.81	0.182	0.192	0.160	0.178
Mizpah.....	1.89	1.55	1.47	1.64	0.071	0.046	0.063	0.060

Nitrogen in volatile matter, in per cent

Hamel.....	2.95	2.74	2.81	2.83	3.30
Taylor's Falls.....	2.99	2.73	2.92	2.87	3.31
Mizpah.....	2.34	2.02	2.39	2.25	2.16

TABLE 4
Weight of forest floor and various constituents
 Pounds per acre

	WOOD I	WOOD II	WOOD III	AVERAGE
--	--------	---------	----------	---------

Weight of dry forest floor

Hamel.....	50,094	55,757	25,265	43,705
Taylor's Falls.....	40,511	41,818	39,204	40,511
Mizpah.....	60,548	46,609	193,406	100,188

Volatile matter

Hamel.....	25,949	31,948	15,816	24,200
Taylor's Falls.....	25,198	26,680	24,424	25,434
Mizpah.....	48,862	35,609	119,138	67,870

Nitrogen

Hamel.....	765	878	444	696
Taylor's Falls.....	752	727	713	731
Mizpah.....	1,145	722	2,847	1,571

Nitrogen in surface 6 inches of soil

Hamel.....	2,823	2,730	2,319	2,624
Taylor's Falls.....	2,104	2,265	2,141	2,170
Mizpah.....	913	613	828	785

Nitrogen in forest floor and surface 6 inches of soil together

Hamel.....	3,588	3,608	2,763	3,320
Taylor's Falls.....	2,856	2,992	2,854	2,901
Mizpah.....	2,057	1,335	3,675	2,356

Proportion of above nitrogen carried in the forest floor, expressed as per cent

Hamel.....	21.3	24.3	16.1	20.6
Taylor's Falls.....	26.3	24.3	25.0	25.2
Mizpah.....	55.6	54.1	77.5	62.4

Phosphoric acid

	HAMEL	TAYLORS FALLS	MIZPAH
a. In forest floor.....	135	125	304
b. In surface 6 inches of soil*.....	1799	1853	999
c. In forest floor and surface soil.....	1934	1978	1303
d. Percentage of whole present in forest floor...	6.9	6.3	23.3

* Total amount as determined by ordinary rock analysis.

lent to 12.6 tons and the last to 96.7 tons per acre. The average for the Hamel and Taylors Falls woods is 21.0 tons per acre. Although the average is higher for Mizpah than for either of the other groups, there was less forest litter in II at Mizpah than in either I or II at Hamel, the high average being due to the very thick floor in III.

Color

The forest floor showed a brownish color at all three places but with a reddish tint at Mizpah and a gray one at Hamel.

TABLE 5

Organic carbon and phosphoric acid in the forest floor and the underlying 6 inches of soil

	HAMEL	TAYLORS FALLS	MIZPAH
Organic carbon:			
a. In forest floor per cent	30.77	33.15	39.47
b. In surface soil per cent	2.66	2.25	0.85
Organic matter, computed (organic C \times 1.724):			
a. In forest floor per cent	53.10	57.10	68.10
b. In surface soil per cent	4.58	3.88	1.46
Ratio of volatile matter to organic carbon:			
a. In forest floor	1.86	1.89	1.85
b. In surface soil	2.25	2.39	3.26
Ratio of organic carbon to nitrogen:			
a. In forest floor	19.0	18.4	24.1
b. In surface soil	13.1	12.3	13.5
Ratio of volatile matter to computed organic matter:			
a. In forest floor	1.1	1.1	1.1
b. In surface soil	1.3	1.4	1.9
Phosphoric acid (P_2O_5):			
a. In forest floor per cent	0.309	0.309	0.303
b. In surface soil per cent	0.125	0.140	0.060
Ratio of organic carbon to phosphoric acid:			
a. In forest floor	100	107.0	130.0
b. In surface soil	21	16.0	14.0

Acidity

The Hamel and Taylors Falls floors showed no acidity and those from Mizpah but little. However, it should be pointed out that all were from woods in which the underlying surface soil showed very little acidity (table 3).

Volatile matter

The volatile matter in the samples, as collected, varied from 51.8 to 80.7 per cent, with an average of 57 at Hamel, 63 at Taylors Falls, and 73 at Mizpah. It is of interest that the sample from the Mizpah wood that showed

much the greatest weight per unit area also carried much the highest proportion of mineral matter. This provides a good illustration of the importance of determining the volatile matter, as well as the dry weight per unit area, in order to compare the amounts of organic matter found in different woods or reported by different investigators. When one, in attempting to include the last trace of leaf mold, scrapes off the mineral soil very closely, he is apt to include so much of the latter that the proportion of volatile matter will be appreciably lowered.

On account of the influence of the included mineral soil upon the composition of the samples—the proportion of this differing with different workers and from time to time—it is desirable to express the percentages and weights on the basis of the volatile portion as well as of the whole of the forest floor.

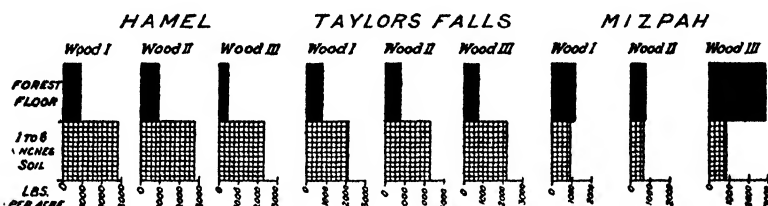


FIG. 2. AMOUNT OF NITROGEN PER ACRE IN THE FOREST FLOOR AND IN THE UNDERLYING 6 INCHES OF SOIL

• Nitrogen

Nitrogen varied from 1.47 to 1.89 per cent, both extremes being found in the Mizpah group. Computed for the volatile portion, the nitrogen varies from 2.02 to 2.99 per cent, averaging much alike at Hamel and Taylors Falls—2.83 compared with 2.87 per cent—but being considerably lower in the Mizpah woods—varying from 2.02 to 2.39 per cent. In the surface 6 inches of soil the percentage of nitrogen is only from one-seventh to one-thirty-fourth as high as in the overlying forest floor.

The amount per acre of nitrogen in the floor varies from 444 pounds in III at Hamel to 2847 in III at Mizpah (fig. 2). Although the highest two values occur at Mizpah and the lowest at Hamel, the other six are very similar. A corresponding variation is to be observed in the amounts per acre of volatile matter. When the total amount of nitrogen contained in the floor and in the surface 6 inches of soil is considered, it is seen that in the deciduous woods the former carries only from 16 to 26 per cent but in the coniferous forest from 54 to 77 per cent. Accordingly the complete burning of the forest floor in one of the southern woods would liberate only about one-fifth of the total amount of nitrogen present, but in the northern forest, half or more, thus making the loss far more serious, provided that the nitrogen in both were at once, or eventually became, equally available, an assumption unproved but still quite probable.

Organic carbon

Organic carbon is highest—39.47 per cent—in the floor at Mizpah, and lowest—30.77—in that from Hamel, although the average nitrogen content in these two groups is practically alike. The organic matter, computed from the organic carbon, amounts to 68.1 per cent in the Mizpah litter, compared with only 53.1 at Hamel. The weight per acre of organic matter, computed from the organic carbon, is about one-eleventh less than the weight of volatile matter. This difference is probably due to the presence of so-called *water of constitution*, which is driven off while the organic matter is undergoing oxidation, and may be due in part to the factor used—1.724—being too low. This difference between the computed organic matter and the determined volatile matter is not serious, but much more weight should be attached to the former than to the latter.

Ratio of organic carbon to nitrogen

In the surface 6 inches of soil in these virgin woods, the ratio of organic carbon to nitrogen varies only between narrow limits, averaging 13.0. It is much higher, however, in the overlying floor, varying from 18 to 24 in the three groups of woods.

Phosphoric acid

In phosphoric acid content all three group composites are alike, carrying 0.30 to 0.31 per cent. At Hamel and Taylors Falls the weight of the phosphoric acid carried in the floor, being only about one-sixteenth of the total, is low compared with that in the underlying soil, but at Mizpah it forms about one-fourth. The effect of burning off the floor may be very marked on soils poorly supplied with readily available phosphoric acid, especially with crops responsive to phosphate applications, for the phosphate left behind will be largely in a form readily available for plants.

Moisture equivalent

The moisture equivalent is high for all the samples of floor, with a maximum of 120.2, a minimum of 97.0, and an average of 110.1, contrasted with 26.0, 13.8, and 20.2 for the underlying surface 6 inches of soil. Assuming that the water-retaining capacity of both floor and soil is equal to the moisture equivalent (1, p. 65), it is evident that after a rain the floor may retain a very large amount of water—almost one inch of rain (0.93 inch) in wood III at Mizpah but only about 0.10 inch in III at Hamel—before allowing any to pass into the underlying soil. As in the forest the moisture content of the floor is never reduced to zero, the amounts of rain which even in its driest condition it could absorb would be considerably below these values.

There is no close connection observable between the percentage of volatile matter in the floor and its moisture equivalent. The samples with the highest

percentages of volatile matter (Mizpah I and II) have the highest moisture equivalents (119.4 and 120.2), and the three at Taylors Falls with similar volatile matter content (62.2 to 63.8 per cent) have similar moisture equivalents (111.4 to 112.8). The sample from III at Hamel, with 62.6 per cent volatile matter, however, has a moisture equivalent of only 97.0, and I at Hamel, with a similar moisture equivalent (97.9) carries only 51.8 per cent volatile matter.

AN EARLIER STUDY

In table 6 are reported data on three samples of forest floor and of the underlying 6 inches of soil collected near Duluth in September, 1915. The one was from virgin coniferous forest on the Duluth Experimental Farm near the city of Duluth, the soil of which is a stony clay loam developed on the Superior lobe of the Late Wisconsin glaciation (6, p. 164-165). Three years later this forest, consisting chiefly of balsam (*Abies balsamea*), was destroyed by the disastrous forest fire of October 12, 1918.

TABLE 6
Forest floor of woods near Duluth

	DULUTH	BLACKHOOF	NICKERSON
Forest floor:			
Nitrogen, <i>per cent</i>	1.48	1.25	1.56
Organic carbon, <i>per cent</i>	39.14	36.16	40.76
Carbon-nitrogen ratio	26.4	29.0	26.1
Weight per square foot, <i>pounds</i>	1.56
Moisture equivalent.. . . .	127.8	122.8	137.7
1 to 6 inches of soil underlying forest floor:			
Nitrogen, <i>per cent</i>	0.17	0.19	0.05
Organic carbon, <i>per cent</i>	2.88	2.49	0.95
Carbon-nitrogen ratio.....	17	13	19
Weight per square foot, <i>pounds</i>	33.0
Moisture equivalent	27.1	34.9	6.4

The site at Blackhoof is 27 miles southwest of Duluth on Superior clay, developed on the lacustral material laid down in glacial Lake Duluth. The forest had originally consisted of a mixture of hardwoods and white pine (*Pinus strobus*), but the latter had been removed some years before the time of sampling.

The third site, that at Nickerson, was 8 miles south of the preceding on a very sandy moraine near the south shore of glacial Lake Duluth. It was covered with jack pine (*Pinus Banksiana*) and red pine (*Pinus resinosa*).

The methods of sampling and of analysis were similar to those described above. The samples are similar in composition and properties to those reported in tables 3, 4, and 5.

SUMMARY AND CONCLUSION

The forest floor was sampled in 9 virgin or nearly virgin Minnesota woods, 6 being remnants of the original southeastern deciduous forest and 3 being in the northern coniferous forest.

The amount per acre was found to vary from 13 to 97 tons, and the content of nitrogen from 1.47 to 1.89 per cent, of volatile matter from 52 to 81 per cent, corresponding to 444 to 2,847 pounds of nitrogen and 8 to 60 tons of volatile matter per acre. The nitrogen in the volatile matter varied from 2.02 to 2.99 per cent, the organic carbon from 31 to 39 per cent, and the ratio of organic carbon to nitrogen from 18 to 24. The samples showed little or no acidity, carried about 0.30 per cent phosphoric acid, and had moisture equivalents of 97 to 120.

A comparison of the weight per acre of nitrogen in the forest floor with that in the underlying 6 inches of soil, showed the one extreme to be in a hard maple wood in which the floor carried only one-sixth as much as the surface soil, and the other in a spruce-balsam-birch forest, in which the floor carried more than three times as much nitrogen as the surface 6 inches of soil.

REFERENCES

- (1) ALWAY, F. J., AND McDOLLE, G. R. 1917 Relation of the water-retaining capacity of a soil to its hygroscopic coefficient. *Jour. Agr. Res.* 9: 27-71.
- (2) Committee on Terminology of the Society of American Foresters and of the Canadian Society of Forest Engineers 1917 Report on Forest Terminology. *Jour. Forestry* 15: 68-101.
- (3) HARMER, P. M. 1926 Minnesota glacial soil studies: III. Density of the surface foot in forest and prairie on the Late Wisconsin Drift. *Soil Sci.* 23: 73-80.
- (4) KITTREDGE, J., JR. 1925 A proposed classification of the forest types of the lake states. *Jour. Forestry* 23: 890-895.
- (5) RAMANN, E. 1911 *Bodenkunde*, ed. 3. Berlin.
- (6) ROST, C. O., AND ALWAY, F. J. 1921 Minnesota glacial soil studies: I. A comparison of soils on the Late Wisconsin and Iowan Drifts. *Soil Sci.* 11: 161-200.

PLATE 1

ILLUSTRATIONS SHOWING CHARACTER OF WOODS AT HAMEL AND TAYLORS FALLS

FIG. 1. Wood II of the Hamel group. The cordwood, shown in the foreground, had been cut between the time of sampling and that of taking the photograph, in September, 1919.

FIG. 2. Interior of Wood III at Taylors Falls, with dense growth of ferns in foreground.

FIG. 3. Interior of Wood III at Hamel.



FIG. 1



FIG. 2



FIG. 3

MINNESOTA GLACIAL SOIL STUDIES: III. DENSITY OF THE SURFACE FOOT IN FOREST AND PRAIRIE ON THE LATE WISCONSIN DRIFT¹

PAUL M. HARMER²

University of Minnesota

Received for publication April 23, 1926

INTRODUCTION

In connection with a study of the composition of virgin soils developed upon the till plains of the Late Wisconsin glaciation, the density was determined for each of the four 3-inch sections of the surface foot in six woods and in six prairie fields—remnants of the original prairies. Only where information is available on the density as well as on the composition of the virgin soils is it possible to draw reliable conclusions as to the effect of cultivation on the organic matter and nitrogen content. Comparatively few data on the density of surface soils *in situ* have been reported and most of these have dealt with sections of 6 to 12 inches from fields that were far from virgin. Studies on the relative density of different depths of the surface soil have been reported by Alway and his co-workers (2; 3, p. 221–226; 4).

According to Hilgard (5, p. 107–8) “the loose tilth and humus content of the surface soil will in general cause it to weigh less, bulk for bulk, than the underlying subsoil, even when the latter is more clayey; moreover, the continuous pressure from above will tend to consolidate the subsoil and substrata;” and sandy land may be assumed to have a weight per cubic foot of about 92 pounds, loams, 80, clay land 75, and garden land and woods-earth 69 pounds.

Warrington reports the density of two fields at Rothamsted and one at Woburn (table 1) and mentions that although the density increased with depth to 36 inches, samplings made to a greater depth showed little further increase. That the surface 9 inches of the old pasture was much lighter, and the lower three sections heavier than those from the arable land, he considered “an excellent illustration of the lightening of a soil laid down to grass, brought about chiefly by the accumulation of vegetable residues in the surface soil” (7, p. 46).

¹ Published with the approval of the Director as Paper No. 615, of the Journal Series of the Minnesota Agricultural Experiment Station.

² Formerly Research Assistant, Division of Soils, Minnesota Agricultural Experiment Station; now Research Associate at Michigan State College of Agriculture and Applied Science.

DESCRIPTION OF FIELDS

Twelve fields with virgin soil, forming four groups of three each, were included in this investigation. The groups are located approximately in an east and west line across the state, two on the prairie and two in the deciduous forest, and all on the unmodified till plain of the Late Gray Drift—the Des Moines lobe of the Late Wisconsin glaciation. The Taylors Falls fields are only a few miles from the Wisconsin boundary, the Canby fields are close to the South Dakota line, and the other two groups lie between.

All the Canby and Renville fields were still in prairie at the time of the study—1916—and virgin to the extent that the native sod had never been disturbed nor the fields fenced nor closely pastured, although for a number of years the crop of native grasses had been removed as hay. Their soils were typical of the prairie—black or brownish black, and high in content of organic matter. The composition of these soils will be dealt with in detail in a later paper of this series. The Taylors Falls and Hamel fields, occupied by deciduous forest, have been described in the preceding paper (2).

TABLE 1
Density of three English soils, from data of Warington
Weight per cubic foot

DEPTH	ROTHAMSTED HEAVY LOAM, OLD PASTURE	ROTHAMSTED HEAVY LOAM, ARABLE LAND	WOBURN LIGHT SAND, ARABLE LAND
<i>inches</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1-9	71	89	97
10-18	95	93	104
19-27	100	98	106
28-36	102	101	107

After a field had been decided upon, two sites, on a level uniform area within it, both well removed from large trees, were selected and from these the density samples were taken. The apparatus consisted of a box of heavy reinforced sheet iron, 12 inches long, 6 inches wide, and 12 inches high, inside measurements, without top or bottom. It was made up of four sections, placed one above the other, each 3 inches deep, held together by two bolts passing vertically through small extensions at the ends of each section. These with the aid of thumb nuts held the four sections firmly together. It was found important to have the inner surface as smooth as possible, and to have each section fit squarely against the adjoining sections.

Around the block of soil selected for the density determination a trench was dug 15 inches deep and wide enough to allow free handling of a spade. The block at the beginning measured about 8 by 14 inches horizontally. The apparatus was placed on top and the block trimmed, by means of a large sharp knife, just enough to permit the apparatus to be pressed down over it. Care was necessary in trimming the block to prevent any bulging of the box, while

avoiding all unnecessary trimming. The work was facilitated by the presence of a fair supply of moisture in the soil at the time and by the small number of stones and tree roots. The latter passing through a block of soil presented the most difficult obstacle. Generally the roots encountered were small, although a few an inch or two in diameter were found. Where a root was met with after a block had been partly removed, the apparatus was worked down close to it and the root then trimmed with a very sharp hatchet, just enough to permit it to pass within the box. When a stone protruded from the block, or occupied parts of two adjacent sections, it was removed and weighed, care being taken to estimate the volume of the portion in each section. The proportionate weights were then assigned to the proper sections.

When the box had been lowered until the top was flush with the surface of the mineral soil, the forest floor having been removed, the block was cut from its base, the box tipped onto its side, and the contents were removed from the pit to an oilcloth. Wherever the surface was dry and crumbly, a sheet-iron lid was placed on the top to prevent loss. The lower, irregular surface was supported by one hand, and, as soon as the block had been placed upon its side on the oilcloth, the bottom was trimmed flush with the lower edge of the box and the trimmings were thrown away. A second sheet of oilcloth was then brought under one end of the box, the nuts were loosened, the bolts were withdrawn until the first section was released, and a large, long straight-edge knife was inserted between it and the second. It was found better to cut slightly into the section being removed, rather than squarely along the edge of it. When the soil section had been separated from the rest of the block and its box removed, the surface of the adjacent section was then trimmed flush with the edge of its box and the trimmings were added to the soil from the first section. The question of whether to remove the 1 to 3-inch or the 10 to 12-inch section first was determined by the moisture content, texture, and other conditions of the soil, the looser end being dealt with first. In the prairie fields, as the surface sections were generally well bound together by roots, the 10 to 12-inch section was removed first. In the forest fields it was found better to remove the 1 to 3-inch section first, the lowest being more compact and generally more moist and holding together much better. After the whole of a 3-inch section had been transferred to the second oilcloth, it was carefully mixed and weighed, and a weighed portion was taken and placed in a sack to be sent to the laboratory for a moisture determination. A portion of the remainder was saved for the preparation of a composite sample for chemical analysis. Then the oilcloth was cleaned, placed under the second soil section, and the procedure repeated.

For the laboratory determination, the composite sample was prepared by mixing equal volumes from the corresponding sections from the two sites in a field. The density data reported for each field are the averages from the two sites.

THE DENSITY

The density, expressed as the weight per cubic foot, is reported in table 2. In the comparison of the successive sections from the same field the most strik-

TABLE 2
Density of the different sections
Weight per cubic foot

DEPTH	FIELD I	FIELD II	FIELD III	FIELD I	FIELD II	FIELD III
<i>inches</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
	<i>Canby</i>			<i>Renville</i>		
1-3	55.5	56.2	60.1	46.5	52.4	58.3
4-6	63.9	72.9	69.9	62.5	52.4	62.3
7-9	69.0	79.4	75.8	69.0	53.2	63.5
10-12	71.1	82.9	81.8	71.5	60.4	66.4
Average 1-12	64.9	72.8	71.9	62.4	54.6	62.6
	<i>Hamel</i>			<i>Taylor's Falls</i>		
1-3	48.4	62.2	57.9	45.0	49.7	55.0
4-6	77.0	82.8	80.5	84.5	79.9	84.6
7-9	85.9	93.9	93.4	87.5	90.4	95.7
10-12	85.8	96.5	96.6	97.4	95.2	101.1
Average 1-12	74.3	83.8	82.1	78.6	78.8	84.1

TABLE 3
Relative density of the four sections
The first taken as unity

DEPTH	FIELD I	FIELD II	FIELD III	AVERAGE	FIELD I	FIELD II	FIELD III	AVERAGE
<i>inches</i>								
	<i>Canby</i>				<i>Renville</i>			
1-3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4-6	1.2	1.3	1.2	1.2	1.3	1.0	1.1	1.1
7-9	1.2	1.4	1.3	1.3	1.5	1.0	1.1	1.2
10-12	1.3	1.5	1.4	1.4	1.5	1.2	1.2	1.3
	<i>Hamel</i>				<i>Taylor's Falls</i>			
1-3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4-6	1.6	1.3	1.4	1.4	1.9	1.6	1.5	1.7
7-9	1.8	1.5	1.6	1.6	1.9	1.8	1.7	1.8
10-12	1.8	1.6	1.7	1.7	2.2	1.9	1.8	2.0

ing difference appears in the forest fields, where the surface is much less dense than the sections below. There is a similar but not nearly so marked difference in the prairie fields, as may be seen better from table 3, which gives for

each field the ratio of the density of each of the lower sections to that of the surface, which is taken as unity. In all the fields the density increases, in general, with each increase in depth, from which it may safely be concluded that if successive sections had been taken from a large number of sites in each

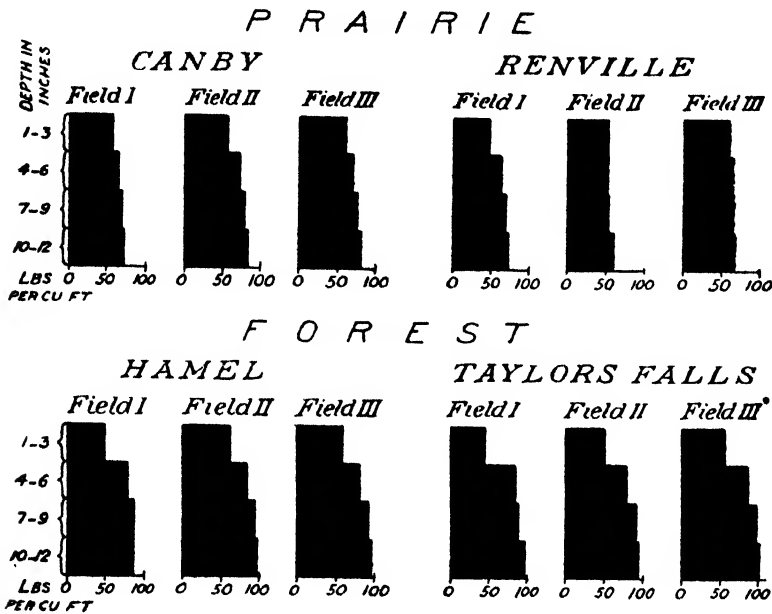


FIG. 1. DENSITIES OF THE FOUR SECTIONS OF THE TWELVE FIELDS

TABLE 4
Density of the different sections of the four groups of fields
Weight per cubic foot

DEPTH	FOREST FIELDS		PRAIRIE FIELDS		AVERAGE FOR FOREST FIELDS	AVERAGE FOR PRAIRIE FIELDS	AVERAGE FOR ALL FIELDS
	Taylors Falls	Hamel	Renville	Canby			
inches	pounds	pounds	pounds	pounds	pounds	pounds	pounds
1-3	49.9	56.2	52.4	57.3	53.0	54.8	53.9
4-6	83.0	80.1	59.0	68.9	81.6	64.0	72.8
7-9	91.3	91.1	61.9	74.8	91.1	68.3	79.7
10-12	97.9	93.0	66.1	78.6	95.4	72.3	83.8
1-12	80.5	80.4	59.9	69.9	80.3	64.9	72.6

field, instead of from only two, an increase with depth would have been found in all the fields. In the forest the increase is more marked than on the prairies.

When the fields of the various groups are considered separately, the fields of each group show considerable uniformity (fig. 1). The lower densities

of Field I at Canby, Field II at Renville, and Field I at Hamel are associated with a higher organic matter content.

The average densities for the different groups are given in table 4. In the surface section there is no distinct difference between prairie and forest.

TABLE 5
Moisture equivalents of the soils of the different fields

DEPTH	FIELD I	FIELD II	FIELD III	AVERAGE	FIELD I	FIELD II	FIELD III	AVERAGE	AVERAGE
<i>inches</i>									
	<i>Canby</i>				<i>Renville</i>				<i>Prairie Fields</i>
1-3	36	30	27	31	39	39	36	38	34
4-6	31	28	26	28	33	34	33	33	31
7-9	29	26	25	27	30	33	31	31	29
10-12	28	25	25	26	28	31	30	30	28
	<i>Hamel</i>				<i>Taylor's Falls</i>				<i>Forest Fields</i>
1-3	30	28	25	28	23	25	25	24	26
4-6	22	20	16	19	16	16	17	16	18
7-9	20	18	14	18	14	14	16	15	16
10-12	20	19	15	18	13	14	15	14	16

TABLE 6
Nitrogen in the different sections

DEPTH	FIELD I	FIELD II	FIELD III	FIELD I	FIELD II	FIELD III
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	<i>Canby</i>			<i>Renville</i>		
1-3	0.39	0.40	0.38	0.50	0.42	0.42
4-6	0.29	0.29	0.25	0.35	0.36	0.31
7-9	0.23	0.23	0.18	0.27	0.33	0.26
10-12	0.19	0.18	0.13	0.19	0.28	0.21
	<i>Hamel</i>			<i>Taylor's Falls</i>		
1-3	0.33	0.29	0.27	0.29	0.32	0.25
4-6	0.13	0.09	0.08	0.08	0.07	0.07
7-9	0.09	0.05	0.05	0.03	0.04	0.04
10-12	0.07	0.06	0.04	0.03	0.03	0.04

The values for Taylor's Falls and Hamel are very similar but those for Canby are higher than those for Renville.

The soil of the prairie fields is much finer in texture as indicated by the moisture equivalents (table 5) but the magnitude of the moisture equivalent de-

depends upon the proportion of organic matter as well as upon the texture of the mineral portion. In the first foot the prairie fields are much the richer in organic matter, as indicated by the nitrogen determinations (table 6). Hence the evidence in hand does not permit the author to determine to what extent the lower density of the prairie soil is due to either the higher proportion of organic matter or the larger proportion of fine soil particles, or to the influence of differences in climate and plant cover.

Although the difference in the densities of the forest and prairie fields may be due to some extent to differences in texture, it probably is more largely due to differences in the content of organic matter. In the forest the organic matter is largely confined to the forest floor and the first two or three inches of soil under the leafmold, with a consequent lesser density of the 1 to 3-inch section, which may explain the sharp increase in density found in passing to the lower sections. In the prairie, on the other hand, where the organic matter content is largely derived from the grass roots, which penetrate for some distance below the surface foot, there is a higher content of organic matter and this is more uniformly distributed, resulting in a very gradual increase in density from the surface downward.

TABLE 7
Weight per acre of surface soil, computed for various depths*

DEPTH	PRAIRIE			FOREST		
	Canby	Renville	Average	Hamel	Taylors Falls	Average
<i>inches</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
1-6½	1,551,000	1,359,000	1,445,000	1,664,000	1,709,000	1,687,000
1-7	1,640,000	1,431,000	1,536,000	1,778,000	1,814,000	1,796,000
1-8	1,912,000	1,655,000	1,784,000	2,110,000	2,144,000	2,127,000
1-9	2,188,000	1,887,000	2,038,000	2,476,000	2,440,000	2,458,000

* By curve interpolation.

In computing the amounts of plant nutrients per acre of land it is most customary to assume 2,000,000 pounds for the surface soil—the portion turned by the plow. This depth is most commonly placed at 6½ inches, as suggested by Hopkins (3, p. 59) although some place it at 7 and others at 8 inches. For constituents which vary little with depth, such as potash, the assumption of arbitrary depth and weight will not seriously affect comparisons, but with the nitrogen and organic matter it is quite different, as with these any depth of sampling other than that necessary to give 2,000,000 pounds per acre will cause considerable error. This depth varies, being 7.5 inches for the Hamel group, 7.7 for that at Taylors Falls, 8.3 inches for that at Canby, and 9.5 at Renville (table 7).

SUMMARY

The density of the soil *in situ* was determined in the four 3-inch sections of the surface foot of twelve Minnesota virgin fields developed on the till

plains of the Des Moines lobe of the Late Wisconsin drift. Six of these fields were in the deciduous forest and six on the open prairie. In the surface section the density of the soil was much alike in prairie and forest, but below that it increased gradually in the former but rapidly in the latter, being one-third the higher in the lower two sections of the forest soil.

REFERENCES

- (1) ALWAY, F. J., AND FILES, E. K. 1912 On the sampling of cultivated soils. *Neb. Agr. Exp. Sta. Ann. Rpt.*, 1912: 52-55.
- (2) ALWAY, F. J., AND HARMER, P. M. 1926 Minnesota glacial soil studies: II. The Forest floor on the Late Wisconsin Drift. *Soil Sci.*, 23: 57-71.
- (3) ALWAY, F. J., AND MCDOLE, G. R. 1916 The loess soils of the Nebraska portion of the transition region: I. Hygroscopicity, nitrogen and organic carbon. *Soil Sci.* 1: 197-238.
- (4) ALWAY, F. J., AND TRUMBULL, R. S. 1912 On the sampling of prairie soils. *Neb. Agr. Exp. Sta. Ann. Rpt.*, 1912: 35-51.
- (5) HILGARD, E. W. 1907 Soils. New York.
- (6) HOPKINS, C. G. 1910 Soil Fertility and Permanent Agriculture. Boston.
- (7) WARINGTON, ROBERT 1900 Physical Properties of Soil. Oxford.

BOOK REVIEW¹

Enzymes. By SELMAN A. WAKSMAN, Associate Professor of Soil Microbiology, Rutgers University; Microbiologist, New Jersey Agricultural Experiment Station; and WILBURT C. DAVISON, Associate Professor of Pediatrics, Johns Hopkins University, School of Medicine; Associate Pediatrician, Johns Hopkins Hospital. The Williams & Wilkins Company, Baltimore, 1926. Pp. xii + 350, bibliog., illus. 15 x 22.5 cm. Price \$5.50.

The vast amount of material that has been accumulated in recent years as the result of studies in the field of enzymes makes the writing of a condensed presentation a difficult matter. The authors have succeeded admirably, however, in making the important results and laboratory methods available in a concise form.

The material has been arranged in four main sections. In section A, Properties of Enzymes, special attention is given to the physical chemistry of enzyme reactions and the factors which influence them. Enzymes of the human and animal body receive the greatest share of attention in section B, Distribution of Enzymes, and this part of the book should be of particular interest to workers in the medical field, to animal physiologists, and to biochemists. Almost one-half of the book is devoted to section C, Methods for the Preparation and Study of Enzymes. In this section, the enzymes are grouped according to the substrate on which they act, and methods of preparation, distribution, optimal conditions, and methods of measurement are given. The detailed directions of this section will be of value to laboratory workers in this field. As compared with the other divisions of the book, section D, Practical Applications of Enzyme Activity, receives only brief attention. The treatment is sufficient, however, to impress one with the extent to which enzyme reactions are applied in the industrial field, and suggestions are made as to possible future applications.

The book is replete with references to the literature, of which over thirteen hundred are listed at the end of the book, in a bibliography arranged alphabetically as to authors and also according to reference numbers in the text. This feature of the book will be of special value to research workers. The arrangement of the book is such that the material is easily accessible, the detailed outline of the table of contents appearing as paragraph headings. The book is thus very suitable for ready reference.

The conciseness of presentation has made the style somewhat abrupt in parts of the book, and for this reason it is not so readable as a more extensive

¹This review was prepared by Prof. Walter C. Russell, of the New Jersey Agricultural Experiment Station.

work might be. Throughout, the emphasis is on facts and practise rather than on theory, and the treatment is too condensed to be of a critical nature.

The extensive bibliography, the brief theoretical treatment, and the wealth of laboratory methods should commend this book to workers who have to deal with enzyme reactions.

..

THE INFLUENCE OF BORON ON THE GROWTH OF THE SOYBEAN PLANT¹

GILBEART H. COLLINGS²

New Jersey Agricultural Experiment Stations³

Received for publication April 19, 1926

INTRODUCTION

Widespread interest was shown a few years ago in the influence on plant growth of fertilizers carrying boron. Because of that interest, this investigation was made to study carefully the influences of boron on the growth of the soybean plant with particular attention to the detection of all toxic and stimulating actions of the element.

As early as 1857 Wittstein and Apoiger (26) reported the presence of boron in the seed of Abyssinian Saoria (*Maessa picta*). Callison (9), in 1890, found the element to be present in fifty-one miscellaneous plants. From time to time other investigators have reported its presence in various plants and plant parts, so that at the present time its distribution in the plant kingdom is known to be almost universal.

The experiment stations of this country conducted many field tests during, and immediately after, the World War to determine to what extent borax might be toxic to such field crops as cotton, corn, and potatoes. This work was necessary because of the widespread damage to field crops by certain American potash fertilizers that contained borax. Toxicity to plants was amply demonstrated when boron in the form of borax was applied in comparatively large quantities. In certain cases, such as in the results secured by Neller and Morse (19), small quantities of boron appeared to stimulate plant growth. In most of this work, however, especially in those cases where the experiments were carried on under field conditions, no minute study of the physiological influences of boron on plant growth has been attempted.

The function of boron in the plant, if it has any, is problematical. Warington (25) of the Rothamstead Experiment Station believes that the action of boron is "probably nutritive rather than catalytic;" thus she accepts its presence as an essential element for at least the broad bean plant.

Experimental procedure and general methods

The influence of boron on the growth of soybeans, and its relative influence when applied in the form of boric acid, potassium borate, and sodium borate, or borax, was studied

¹ Thesis presented to Rutgers University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1924. Paper No. 320 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

² Grateful acknowledgment is hereby made to Dr. J. G. Lipman, Prof. A. W. Blair, and Dr. John W. Shive, for their advice, coöperation, and encouragement throughout the course of this piece of research.

³ Now Associate Professor of Agronomy at Clemson College, S. C.

under five heads: first, on germination; second, on the seedling phase of growth of soybeans in nutrient solutions; third, on plants grown to maturity in nutrient solutions; fourth, on plants grown in sand; and fifth, on plants grown in soil.

The Manchu variety of soybeans (*Soja max*) was used throughout the experiments. In order to secure the greatest uniformity among the plants grown, a pure line strain of the variety was used. The seed were hand graded and were planted in moist sphagnum moss. In about one week after planting, selected seedlings were transplanted to the culture jars or pots, which contained the solutions, sand, or soil.

An excellent grade of seashore fine sand, secured from a pit, was used in all the sand cultures. The same sand was used in all the germination experiments, but it was first thoroughly washed free of all clay particles.

The soil used in the soil cultures was Norfolk coarse sand secured in the vicinity of Milltown, New Jersey. The soil used in the germination experiments was Sassafras loam, secured on the farm of the New Jersey Agricultural Experiment Station.

For most of the pot culture work, glazed earthenware pots of a capacity of about two kilos of dry sand were used. Into each pot was weighed 1700 gm. of dried sand or soil. All pots were tared with coarse washed gravel.

To each pot of the sand and soil cultures, a complete fertilizer, consisting of 0.187 gm. of chemically pure ammonium sulfate, 0.75 gm. of acid phosphate, and 0.25 gm. of chemically pure potassium chloride, was applied. The acid phosphate was thoroughly mixed with the dry sand or soil, but the potassium chloride was first dissolved in water and one-half added just before the seedlings were transplanted, and one-half about three weeks later. The ammonium sulfate was also dissolved in water and applied in three weekly installments. The first applications of ammonium sulfate and of potash were made at the same time.

The culture jars used in the water culture work were of colorless glass, and had a capacity of 900 cc. (1 quart) and 1800 cc. (2 quarts). These jars had for some years been used for culture work, and were, therefore, thoroughly seasoned.

Three seedlings were transplanted to each jar or pot. For the water culture jars, the three seedlings were mounted in a double-piece paraffined cork stopper, such as that devised by Tottingham (23) and placed in each culture jar. Cylindrical shells of manila paper were placed around the jar to exclude the light from the plant roots. These paper shells were also used around all reservoirs of solutions to prevent the growth of algae, and to prevent such chemical action of light as the conversion of nitrates to nitrites as has been reported by Baudish (4).

After the application of the boron to the sand and soil cultures, the moisture was maintained at 12.5 per cent of the dry weight of the sand or soil by weighing once each day and replacing the loss of weight with distilled water. Similarly, all germination tests conducted in sand were maintained at 12.5 per cent water by weighing once each day. Two weighings were sometimes made on hot days when excessive evaporation was taking place.

A modification of Tottingham's four-salt solution $T_1 R_1 C_1$, as devised by Jones and Shive (15) was used in all of the water culture work. This solution is made up of $(NH_4)_2SO_4$, KH_2PO_4 , $Ca(NO_3)_2$, and $MgSO_4$ in volume molecular proportions of 0.0014, 0.00211, 0.00146, and 0.01659 respectively. The stock solutions of these chemicals were made up to one-half molecular concentrations.

Iron was added to the water cultures in the form of soluble ferric phosphate in amounts equivalent to about 0.25 mgm. per liter of the nutrient solution. The ferric phosphate was made up in concentrations equivalent to about 1 mgm. of iron per cubic centimeter of solution. This stock solution was always prepared just before being added to the culture solutions. This element was added to the culture solutions when the plants appeared to need it.

In order to incorporate different amounts of boron in each culture solution, it was found necessary to make up each culture separately, although the same nutrient solution was used in all the water culture work.

All cultures of all series of plants grown during the seedling phase were constantly re-

newed according to a drip and drain method somewhat similar to that devised by Allison and Shive (2). One quart of solution was allowed to pass through the system every twenty-four hours. From time to time the glass siphons, which were exposed to light, were washed in acid and then in distilled water to free them from algae.

In most cases, duplicate series were grown at the same time. In such cases the corresponding cultures of each series were placed adjacent to each other, in order that environmental conditions might be as nearly alike as possible. Weekly records were kept of all changes taking place in the uniformity of the tops of each series, and of the roots of all plants grown in water cultures. When the growth period of each series was completed, the tops were severed from the roots and a determination of the green weight of both tops and roots

TABLE 1
Influence of boric acid, potassium borate, and borax on germination

BORON PER LITER OF SOIL SOLUTION	PERCENTAGE OF GERMINATION AT INTERVALS AFTER SEEDLINGS APPEARED*											
	Sand media									Soil (sassafras loam) media		
	Boric acid			Potassium borate			Borax			Borax		
	Second day	Fourth day	Sixth day	Second day	Fourth day	Sixth day	Second day	Fourth day	Sixth day	Second day	Fourth day	Sixth day
mgm.												
0	80	85	87	75	85	90	65	70	70	75	77	80
0.5	92	97	97	72	77	77	80	80	80	90	92	92
1 0	85	90	90	82	90	90	62	80	80	82	85	85
10 0	82	97	100	70	75	82	57	67	67	77	82	85
25.0	52	75	82	70	75	77	30	45	45	72	82	82
50.0	50	70	72	45	77	77	22	32	32	70	70	72
100.0	22	30	40	5	32	45	5	10	10	80	80	80
150 0†	22	32	35	15	15	17	10	12	20			
200 0†	12	15	20	7	15	15	0	5	5			
250 0	0	0	0	0	0	0	0	0	0	87	95	95
500.0	0	0	0	0	0	0	0	0	0	52	55	57
1000 0	0	0	0	0	0	0	0	0	0	30	40	40

* Average of two tests.

† These two concentrations were used in another experiment at a later date.

was made at once. The roots were first gently rinsed with distilled water and then placed on blotting paper for a few minutes before weighing. Both the roots and tops were placed in manila bags and allowed to dry at ordinary temperature in the greenhouse. They were then placed in an oven and kept at 100° C. for 24 hours. The contents of each bag were then weighed and the dry weight of each culture was determined.

THE INFLUENCE OF BORON ON GERMINATION

The germination experiments were made in No. 2 enamel pans. Into each pan, 200 gm. of dry washed sand, or in one experiment, of dry Sassafras loam soil, was weighed and leveled. Twenty hand-selected seed were uniformly distributed over the surface and an additional 800 gm. of dry washed sand or of the dry soil was added. Into each pan was then poured 125 cc. of a solution containing the desired amount of boron.

Records were taken for six successive days after the first seedlings appeared above the surface of the sand or soil. The number and appearance of the seedlings were recorded each day. All germination experiments were run in duplicate.

Experiments conducted in washed sand

The results of all experiments conducted in washed sand are given in table 1. It will be observed from these data that 250 mgm. of boron per liter, in the soil (sand) solution of thoroughly washed sand either in the form of boric acid, potassium borate, or borax, is capable of entirely stopping germination.

A half century ago Heckel (13) reported the injurious effects of alkali borates on the germination of seed, but the milligram concentration of the soil solution that will prevent germination of soybeans has never before been determined. The data in table 1 show that the seeds vary in their resistance to this injurious element. A few of the seed were able to resist the toxic action of the element in concentrations as high as 200 mgm. per liter of the soil (sand) solution. In all these experiments at least 100 mgm. of boron was capable of reducing materially the percentage germination of the seed. It might well be observed at this time that the percentage germination secured in all the check tests, 82.5 per cent, was very good for soybeans. The weaker concentrations of boron appear to delay germination. It was observed that, for the first few days after the seedlings began to appear at the surface of the sand, 10 mgm. of boron per liter of soil solution was capable of delaying germination.

Many investigators have noted this retarding effect of boron compounds on the germination of seeds, particularly under field conditions. Voelcker (24) has noted this effect of boric acid and borax on wheat and barley. This same influence of borax on cotton has been reported by Blackwell and Collings (5) and by Skinner and Allison (22), on cotton and corn by Sherwin (21), on corn and potatoes by Blair and Brown (6) and by Neller and Morse (19). Archangeli (3) tested the influence of boric acid on germination by soaking a large number of species of seeds in concentrations of boric acid containing about 1773, 886, and 443 mgm. of boron per liter respectively. He reported that 1773 mgm. prohibited germination altogether, and that the other concentrations delayed germination in proportion to the concentration. In 1892 Morel (18) concluded that boric acid and borax when used in equal quantities, equally diminished or inhibited the germination of kidney beans and wheat when the seed were soaked in solutions of each.

This latter conclusion is not upheld by the results of this work with soybean seed. Observation and a careful study of table 1, indicate that in their action on germination boron compounds are not equal when used quantitatively, but that they are about equal when compared on a boron content basis.

In these germination experiments wherever as much as 10 mgm. of boron was applied per liter of soil (sand) solution the edges of the young leaves were tinged yellow; with the higher concentrations of boron the entire seedlings were chlorotic. Warington (25) called attention to a similar condition with the broad bean, but she observed that the condition appeared to be overcome with further growth of the plants.

Experiments conducted in soil

It has been noted by many that under field conditions the injury to field crops from borax-carrying fertilizers is greater in sections where the soils are sandy. This, as has been pointed out by Cook and Wilson (11), is probably due to the absorption of the boron by the colloidal material of the heavier soils, or to a chemical conversion of the soluble boron compounds into insoluble ones. In order to determine the extent of this fixation of boron, a germination experiment was made on Sassafras loam soil. Borax was used as the carrier of boron. Table 1 gives the results of this experiment also. These data show that a minimum equivalent of about 500 mgm. of boron per liter of soil solution materially inhibited seed germination in soil. A minimum of 250 mgm. of boron produced an unhealthy appearance in the seedlings. Germination was not entirely prevented by 1000 mgm. of boron per liter of soil solution. A comparison of these results with those secured with washed sand, indicates considerable dilution of the boron through absorption or chemical combination.

A close examination of the seed that failed to come up revealed that the growing point of the plumule had been killed. Where 1000 mgm. of boron was present in the soil solution of the experiments conducted in sand, practically no development of the plumule had taken place, but with the weaker concentrations of boron, the plumule showed greater development. Likewise, the extent of growth was found to be correlated with the concentration of the boron. The development of the root systems of the seedlings in the pans receiving 10 mgm. and more of boron was diminished by increasing the concentration of boron.

It is not known just what physiological effect boron has on the germinating seed, or on the young seedling. Schreiner et al. (20) have suggested that the element may interfere in some way with the liberation of sugars. They call attention to the fact that borax is capable of forming a chemical union with sugar alcohols, such as mannite. It is upon this combination that one of the present methods of determining borax is based.

A brown incrustation was noticed on the surface of all pans of sand, and a gray incrustation on all pans of soil receiving 500 mgm. or more of boron per liter. Warington (25) made a similar observation when 4 gm. of boric acid was added to 22.5 pounds of soil. She suggested that this was due to a surface concentration of the boron compounds. This suggestion is probably correct, for in the experiments reported in this paper where washed sand was used, no other soluble matter could have been present.

THE INFLUENCE OF BORON ON SOYBEANS GROWN IN SAND

After the sand had been weighed into the pots and the fertilizer added, the young seedlings were transplanted to the pots and a few days later solutions of the boron compounds were added to each culture.

Six series of nine cultures each were run. These will be designated as Boric

Acid Series I and II, Potassium Borate Series I and II, and Borax Series I and II. Anhydrous borax or the borax equivalent of boric acid or of potassium borate, was applied on an acre basis. As the moisture was maintained at 12.5 per cent, the concentration of boron in the soil (sand) solution could be determined. The applications of boron varied from 1 to 75 pounds per acre, or the equivalent of 0.46 to 34.53 mgm. per liter of the soil (sand) solution. The individual applications on a pound per acre basis, and on a milligram of boron per liter of soil (sand) solution basis, are given in table 2.

Toxicity of boron as shown by visible injury to the leaves

Within from three to five days after the boron had been added, visible injury occurred on all plants receiving an initial application equivalent to 10 pounds or more of borax per acre. An application of 10 pounds of borax per acre

TABLE 2
Cultures showing boron injury on the leaves of plants grown in seashore sand

AMOUNT OF BORAX OR EQUIVALENT PER ACRE	AMOUNT OF BORON PER LITER OF SOIL SOLUTION	TIME ELAPSING BETWEEN APPLICATION OF BORON COMPOUND AND APPEARANCE OF INJURY					
		Boric Acid Series		Potassium Borate Series		Borax Series	
		I	II	I	II	I	II
pounds	mgm.	days	days	days	days	days	days
0	0						
1.0	0 46	17	17	21	21	17	17
2.5	1.15	10	10	10	10	11	11
5.0	2 30	9	9	9	9	10	10
7.5	3 45	9	9	6	6	9	9
10.0	4 60	6	6	5	5	5	5
25.0	11.51	4	4	4	4	4	4
50.0	23 02	3	3	4	4	3	3
75.0	34 53	3	3	3	3	3	3

produces a concentration of boron in the soil (sand) solution—provided none is fixed—of 4.6 mgm. per liter of cultural solution. Table 2 also gives the number of days elapsing between the application of the boron compound and the appearance of injury in the boric acid, potassium borate, and borax series. It will be observed that the visible injury to the leaves occurred almost simultaneously in the boric acid, potassium borate, and borax series. Furthermore, the injury to the leaves appeared practically simultaneously in all cultures receiving the same application of boron. In all six series that received more than 50 pounds of borax, or its equivalent per acre, all plants were killed within a week after the application of the boron compounds. The plants of these cultures did not show very clearly the characteristic leaf injury. The concentration of the toxic element was apparently so great in these cultures that the entire plant turned chlorotic and died within a short time. This

was also observed later when large applications of boron were made in the water culture experiments. Wherever 25 pounds of borax per acre was applied, the plants were nearly dead when harvested. The injury appeared consecutively from higher to lower applications until an application of 1 pound per acre, or the equivalent of 0.46 mgm. of boron per liter of soil (sand) solution, produced the toxicity on the leaves. No application lower than 1 pound per acre was made. The extent of the visible injury was about the same in all cultures receiving the same applications of boron, whether in the form of boric acid, potassium borate, or borax. All cultures receiving more than 7.5 pounds of borax per acre or 3.45 mgm. of boron per liter of soil (sand) solution, showed a correlated stunting of the plants with an increase in the application of the boron compound.

TABLE 3
Dry weight per plant of tops of soybeans grown in seashore sand

POT	AMOUNT PER ACRE OF BORAX OR EQUIVALENT	DRY WEIGHT PER PLANT OF SOYBEAN TOPS FOR*		
		Boric Acid Average Series I and II	Potassium Borate Average Series I and II	Borax Average Series I and II
	<i>pounds</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1	0	1.6361	1.6313	1.2797
2	1 0	1.1366	1.5321	1.3459
3	2.5	1.5450	1.6553	1.1566
4	5 0	1.4399	1.5760	1.4655
5	7.5	1.2454	1.5254	1.2733
6	10 0	1.3476	1.5171	1.1587
7	25 0	1.1087	0.9760	1.1449
8	50 0	0.5068	0.5348	0.4910
9	75.0	0.2619	0.2491	0.3102

* Average of six plants.

The influence of boron on the dry weights of plants grown in sand

The plants were harvested at the end of their seedling phase of growth. The roots were not harvested. Table 3 shows that boron in any form has a very harmful influence on the dry yield of plant tops. The dry weight per plant was reduced by as low an application as 1 pound of boric acid per acre; by 5 pounds or more of potassium borate; and by 7.5 pounds or more of borax. However, the 1.0 pound application of potassium borate and the 2.5 pound application of borax produced less dry matter than their respective checks.

The dry weights of these six series again corroborate the conclusions secured with the germination test: first, that the borate ion produces the injury; and, second, that compounds of boron are toxic in proportion to the boron they carry, and such compounds are comparable on that basis. It is apparent that the amount of absorption of boron is very small in pure sand, for injury was secured here with 0.46 mgm. of boron per liter of soil (sand) solution.

The stimulating influence of boron

There was no evidence from any of the six series that boron is essential or stimulating to the growth of soybeans. The average dry weights of all duplicate series (table 3) reveal only three cases where the dry weights are greater than that of the check—where 1 and 5 pounds of borax, respectively, and where 2.5 pounds of potassium borate were added.

THE INFLUENCE OF BORON ON SOYBEANS GROWN IN SOIL

Since most of the boron injury reported from borax-carrying fertilizers used under field conditions was obtained on sandy types of soil of the Coastal Plain, it was thought best to use a representative Coastal Plain soil—Norfolk

TABLE 4
Cultures showing boron injury on the leaves of plants grown in Norfolk sand

AMOUNT OF BORAX OR EQUIVALENT PER ACRE	AMOUNT OF BORON PER LITER OF SOIL SOLUTION	TIME ELAPSING BETWEEN THE APPLICATION OF BORON COMPOUND AND APPEARANCE OF INJURY					
		Boric Acid Series		Potassium Borate Series		Borax Series	
		III	IV	III	IV	III	IV
pounds	mgm	days	days	days	days	days	days
0	0						
1.0	0 46	22	22	20	20	22	22
2.5	1 15	10	10	10	10	16	16
5 0	2 30	9	9	9	9	7	7
7.5	3.45	7	7	8	8	7	7
10.0	4 60	6	6	5	5	6	6
25.0	11 51	4	4	4	4	5	5
50 0	23 02	3	3	3	3	5	5
75 0	34 53	3	3	3	3	5	5

sand—for this study. It was also believed that with this class of soil the effect of small applications of boron on plant growth would be more pronounced than with a heavier type of soil.

As in the previous study, six series of nine cultures each, were run in this test. These series are designated as Boric Acid Series III and IV, Potassium Borate Series III and IV, and Borax Series III and IV. The same amounts of soil, and the same applications of fertilizer were used as in the experiments with seashore sand.

Toxicity of boron as shown by the visible injury to the leaves

Visible injury to the leaves again occurred in all the cultures of all the series receiving boron. In table 4 is given the number of days elapsing between the application of the boron compound and the appearance of injury. It will be observed that the visible injury occurred in the six series in about the same

length of time. Again, the same concentration of the borate ion appeared to produce the same results, regardless of the carrier.

Tables 2 and 4 show that the results secured with Norfolk sand are similar to those secured with pure sand. In all cases 1-pound applications of borax, or its equivalent, were sufficient to produce visible injury on the leaves. This soil was a coarse sand and carried only small amounts of clay. It is, therefore, not surprising that the results are closely correlated to those secured with pure sand.

Special attention is directed to the small amounts of boron capable of producing visible injury. Although considerable work has been done in pot cultures in connection with field experiments by many investigators, few have indicated that less than 5 pounds per acre of borax was toxic to any plant even when the material was applied in the drill. This is probably because the first

TABLE 5
Dry weight per plant of tops of soybeans grown in Norfolk sand

POT	AMOUNT PER ACRE OF BORAX OR EQUIVALENT	DRY WEIGHT PER PLANT OF SOYBEAN TOPS* FOR		
		Boric Acid Average Series III and IV	Potassium Borate Average Series III and IV	Borax Average Series III and IV
	<i>pounds</i>	<i>gm</i>	<i>gm.</i>	<i>gm.</i>
1	0	1.3841	1 0776	1.1205
2	1 0	1.0200	1 0075	1.8366
3	2 5	0.9610	0.9171	1.0694
4	5.0	0.9057	0.8375	1.0295
5	7.5	0.9432	0.8582	0.9358
6	10 0	0.9156	0.7072	1.0208
7	25.0	0.5600	0.5434	0.6650
8	50.0	0.2983	0.2103	0.4101
9	75.0	0.2864	0.2298	0.2129

* Average of six plants.

appearance of the injury was not readily recognized. It has been observed frequently in these experiments that a plant might appear perfectly sound to a casual observer, and yet upon a close inspection the presence of the visible toxicity on the leaves could be noted. It was also observed that many of the lower leaves of the plant may show the injury, and yet the dry weight of the plant may not be reduced materially.

It is very probable that had a $\frac{1}{2}$ -pound application of borax been made and had the plants been allowed to grow to maturity, toxic injury to the leaves would have appeared before harvesting.

The influence of boron on the dry weights of plants

The average dry weights of all duplicate series are given in table 5. These data show that an application of 1 pound of borax per acre in the form of

boric acid or potassium borate, or of 2.5 pounds per acre in the form of borax, is sufficient to decrease the dry weights below that of the controls during the seedling phase of growth.

The stimulating influence of boron

There is no indication in any soil series of any marked stimulation to soybeans, although as many as 162 plants were grown in these six series. On the other hand, Voelcker (24) has reported a slightly stimulating effect on wheat with quantities of borax not exceeding 27 pounds per acre, and Breckenbridge (7) grew in pots potatoes which appeared to be stimulated with 4 to 6 pounds of borax per acre.

It should not be overlooked that boron compounds are somewhat antiseptic, and that a certain amount of soil sterilization may take place. This action of boron may influence the soil organisms, and thus have an indirect influence on plant growth.

THE INFLUENCE OF BORON ON THE SEEDLING PHASE OF GROWTH OF SOYBEANS IN WATER CULTURES

Although many investigators have reported boric acid and borax to be toxic to plants, very little exact work has been done to determine at what minimum concentrations the toxic compounds will produce the harmful effects. Most of the work that has been reported has been conducted under field conditions. Many investigators have called attention to the fact that where toxic compounds are applied under field conditions various factors may enter in to obscure the results. For example, the amount and distribution of rainfall may alter the concentration of the soil solution and, therefore, influence the relative toxicity of the compound. Even where toxic compounds are applied to soil in pots, and the pots kept under controlled greenhouse conditions, absorption of the toxic compound will take place to an extent depending upon the physical and chemical nature of the soil. This absorption will, in turn, influence the concentration of the toxic element in the soil solution, and thus vary the extent of injury to the plant.

This being true, it naturally follows that water culture tests, rather than pot or field tests, offer the best possible means of studying under controlled conditions the harmful and beneficial influences of minute quantities of chemical compounds upon the growth processes of any plant, provided the plant in question can be grown successfully in nutrient solutions. It was with this idea in mind that this phase of the study was undertaken.

The soybean was used for this study because legumes are more sensitive to toxic compounds than are non-legumes, and because it has proved so well adapted to nutrient solution work under greenhouse conditions. Cook (10) has reported that legumes are more sensitive to boron compounds than non-legumes. The Modified Tottingham solution was used in all the nutrient solu-

tion work because Jones and Shive (15) have reported favorable results when growing soybeans in this solution.

Several investigators have reported the toxic influence of soluble boron compounds on the growth of seedlings. Archangeli (3), for instance, found that seedlings of maize, white lupines, vetch, or wheat placed in a solution of boric acid containing about 1773 mgm. of boron per liter of solution were killed, but that they were less severely injured with weaker concentrations. Likewise, Kahlenburg and True (16), limiting their experiments to from fifteen to twenty hours duration, found that seedlings of lupines put into a solution of boric acid containing about 440 mgm. of boron per liter were killed, concentrations of 220 mgm. of boron per liter left the plants apparently just alive, and a weaker concentration of 110 mgm. produced no injurious influence.

At the beginning of this experiment it was thought probable that any harmful or beneficial influences of boron compounds on soybeans grown in nutrient solution would appear in the early stages of growth. It was also thought probable, that if these influences should manifest themselves when the plants were young, they might be obscured by further growth of the plant. A study was first made, therefore, of the influence of boron compounds on the growth of soybean plants during their seedling phase. A comparative study was also made of the influence of boron in the form of boric acid, potassium borate, and borax.

As no investigator had worked on this problem with the soybean when grown in the Modified Tottingham nutrient solution, it was found necessary first to apply boron to the culture solutions over a wide range of concentrations, and to determine whether any noticeable effects could be produced. Therefore, a series of fifteen cultures receiving boric acid was run and harvested 21 days later when the plants were in full bloom. Boric acid was applied in concentrations ranging from 0.006 to 55.89 mgm. of boron per liter of the nutrient solution. The individual concentrations are given in table 6.

Toxicity of boron as shown by visible injury to the leaves

Five days after the application of the boric acid, small dark brown spots began to appear on the first pair of leaves of culture 15—this culture had received 55.89 mgm. of boron per liter of the nutrient solution. The injury, which was very pronounced, was apparently of the same type as that observed on plants grown in sand and soil containing boron-carrying fertilizers. This injury appeared within three days, consecutively in the next five cultures receiving lower concentrations of boron, and was produced by all applications of more than 0.44 mgm. of boron. It is evident that boron in the form of boric acid in nutrient solution is toxic to soybean seedlings, and that the extent of this toxicity is dependent upon the concentration of boric acid. It was also observed that the appearance of this visible injury on the leaves occurred simultaneously and uniformly on all plants of each culture.

After securing these results, another series, designated as Boric Acid Series VI, was started to determine whether an application of boron as boric acid, in

concentrations ranging between 0.44 and 0.89 mgm., would produce the injury during the same growth period. Visible toxicity to the leaves again appeared. Table 6 also shows the concentrations of boron used in all cultures of this series,

TABLE 6

Amount of boron applied per culture and time elapsing between the application of boron compounds and the appearance of the injury

CULTURE	BORIC ACID SERIES V		BORIC ACID SERIES VI	
	Boron per liter of nutrient solution	Time required for injury to appear	Boron per liter of nutrient solvent	Time required for injury to appear
	mgm.	days	mgm.	days
1	0		0	
2	0.006		0.22	
3	0.013		0.33	
4	0.027		0.44	
5	0.055		0.53	21
6	0.110		0.62	
7	0.220		0.71	20
8	0.440		0.80	19
9	0.890	17	0.89	19
10	1.740	7	1.07	17
11	3.490	7	1.25	17
12	6.980	6	1.43	14
13	13.970	6	1.60	14
14	27.940	5	1.74	13
15	55.890	5	3.49	11

CULTURE	POTASSIUM BORATE SERIES V		POTASSIUM BORATE SERIES VI	
	Boron per liter of nutrient solution	Time required for injury to appear	Boron per liter of nutrient solution	Time required for injury to appear
	mgm.	days	mgm.	days
1	0		0	
2	0.002		0.28	
3	0.004		0.43	21
4	0.009		0.56	19
5	0.018		0.67	19
6	0.036		0.79	17
7	0.072		0.90	19
8	0.140		1.01	14
9	0.280		1.12	14
10	0.560		1.35	14
11	1.120	17	1.58	14
12	2.250	7	1.80	14
13	4.510	7	2.03	14
14	9.030	6	2.25	13
15	18.060	5	4.51	10

the cultures on which the injury occurred, and the time elapsing between the application of the boron compound and the appearance of the injury on the

leaves. It will be observed that at the time this series was harvested, visible injury to the leaves had been produced where only 0.53 mgm. of boron had been applied per liter of the nutrient solution, and that injury had not occurred where 0.44 mgm. of boron had been applied. These results corroborate as closely as could be expected the results secured with Boric Acid Series V.

Simultaneously with Boric Acid Series V and VI, two similar series were run with potassium borate as the carrier of boron. The concentrations of boron, and the time elapsing between the application of the boron compound and the appearance of the injury on the leaves are given in table 6. Again a similar visible injury occurred in those cultures receiving a high dose of boron. It thus appeared that boron, either in the form of boric acid or potassium borate, was capable of producing the injury. At harvest time, the highest applications of boron in this series which had not showed the injury was 0.56 mgm. Potassium Borate Series VI was then run with a smaller variation in the concentrations of boron. Table 6 shows that the lowest concentration producing injury during this test was 0.43 mgm. This slight variation from the results secured in Potassium Borate Series V might be due to the differences in the environmental factors during the periods when the two series of cultures were grown.

It is interesting to note that the lowest concentration of boron in the form of boric acid producing injury during the seedling phase of growth (0.53 mgm.) is very nearly the same as in the potassium borate series (0.43 mgm.). It thus appears that the borate ion produces the visible injury, and that the time required is correlated with the amount of boron present in the culture solution. The absorption of enough boron to produce visible injury on the leaves takes place in less time from the cultures containing the higher concentrations of boron.

Relative influence of boron in the form of boric acid, potassium borate, and borax on visible injury

In order to compare the relative influence of boron in various forms, a series of duplicate cultures was run to determine the time required for the injury to appear, and the relative degree of the injury at any given time in cultures to which the same amounts of boron had been added in the form of boric acid, potassium borate, and sodium borate or borax. The concentration of boron used in all cultures was 3 mgm. per liter of nutrient solution. The plants were grown in sand in percolators to which a continual renewal of the nutrient solution was made.

In this series the injury occurred on all plants simultaneously, both on the first and second pairs of leaves. These results again show that the borate ion produced the toxicity. The extent of the injury, after its appearance up to the time of harvesting, was apparently the same on all plants at all times. Plate 1 shows the type and the extent of the injury at harvest time on the first pair

of leaves of representative plants which received boric acid, potassium borate, and borax, respectively.

The influence of boron on the development of the root system

Warington (25) and Agulhon (1) have reported that some concentrations of boron, added in the form of boric acid to the water cultures, markedly injured, and others stimulated the root systems of plants. In the five series of plants under discussion no visible injury to the root system could be detected at any time except when the application of boron was so great that it killed, or retarded, the growth of the entire plant. In such cases the root systems of the injured plants were smaller, but in other respects similar to those not receiving boron.

The stimulating influence of boron

Agulhon (1) secured an increase in dry weight of wheat plants when boric acid was applied in small quantities ranging from 0.5 to 10.0 mgm. of boron per liter of synthetic culture solution. Boric Acid Series V showed some evidence of stimulation when either the green or the dry weights of tops, roots, or total plant were compared. This evidence, however, was not present in Boric Acid Series VI or in Potassium Borate Series V and VI. The dry weights of these three series showed only normal variations. In no case was there any visible evidence during the seedling phase of growth of any stimulation to the roots or tops due to boron, either in the form of boric acid or of potassium borate. The plants in all control cultures appeared to be perfectly normal.

THE INFLUENCE OF BORON ON SOYBEANS GROWN TO MATURITY IN SOLUTION CULTURES

An analysis of the data already given on the influence of boron on the seedling growth of soybeans indicated that had harvesting been delayed beyond the flowering period, or had the plants been grown to maturity, smaller concentrations of boron than 0.4 or 0.5 mgm. per liter of cultural solution would have produced injury. In order to determine whether this were true, two series of fourteen cultures each were started to which boron in the form of boric acid was added, and three series of fourteen cultures each to which boron in the form of borax was added. It was also desired to determine whether there were any stimulating effects in the advanced phases of growth of the plant, as no marked stimulation had been shown during the seedling phase.

In all of these series the constant renewal method of solution change, as previously described, was employed. The plants of Borax Series IX were grown in sand in percolators. The concentration of boron was so arranged that the lowest producing toxicity during the seedling phase of growth was placed about midway in the series. It was thought that if any stimulating

influence was present it would show itself at a concentration just below or just above that which produced the appearance of the visible injury on the leaves.

Boron was applied to the five series in concentrations ranging from 0.01 to 2.50 mgm. per liter of the cultural solution. The individual applications of boron per culture are given in table 7.

TABLE 7
Cultures showing boron injury on the leaves when plants were grown to maturity

CULTURE	BORON PER LITER OF NUTRIENT SOLUTION	TIME ELAPSING BETWEEN THE APPLICATION OF BORON COMPOUND AND APPEARANCE OF INJURY FOR			
		Boric Acid Series		Borax Series*	
		VII	VIII	VII	VIII
	mgm.	days	days	days	days
1	0				
2	0 01				
3	0 05				
4	0 10				46
5	0.20	31	37	42	40
6	0 30	29	26	39	39
7	0 40	24	26	38	38
8	0 50	28	24	33	34
9	0 60	22	22	25	25
10	0.70	18	18	21	21
11	0.80	17	17	22	21
12	0.90	16	15	20	20
13	1.00	16	16	19	19
14	2 50	11	11	13	13

* Borax Series VII and VIII were run at a different time from Boric Acid Series VII and VIII.

Toxicity of boron as shown by visible injury to the leaves

The same injury noticed during the seedling stage of growth, when boric acid and potassium borate were applied, was noted in all five of these series where boric acid and borax were applied. In about one and a half to two weeks after the application of the boron, injury appeared on the leaves where 2.5 mgm. had been applied. A few days later the injury occurred where 1.0 mgm. had been applied, and from this time on until harvest the injury appeared in successive lower concentrations in all the series. In about four or five weeks the injury had appeared in cultures receiving 0.5 mgm. of boron. In about seven weeks the injury had occurred where as low as 0.2 mgm. of boron had been applied. The plants were harvested at maturity, when they were eight weeks old. The lowest application in Boric Acid Series VII and VIII and in Borax Series VII showing the injury was 0.2 mgm. In Borax Series VIII

and IX, 0.1 mgm. of boron showed the injury. It will be observed that down to a concentration of about 0.6 mgm. the injury in all series occurred simultaneously.

Special attention is directed to the very small quantities of boron that are capable of producing the visible injury on the leaves—particularly small when compared with other substances, such as arsenic and copper salts, which are generally considered poisonous to plants. Hotter (14) has observed that the leaves of peas and maize were injured by applications of boric acid. He was able to detect injury with the equivalent of 1.7 mgm. of boron per liter of nutrient solution, which is about seventeen times as great as that found in this experiment.

Relative influence of boron in the form of boric acid and of borax

Hotter (14) has also reported that weight for weight, borax is less toxic than potassium borate, which in turn is less toxic than boric acid. The results secured in Boric Acid Series VII and VIII, and Borax Series VII, VIII, and IX confirm the results of Hotter. Borax carries less boron than potassium borate, which in turn carries less than boric acid. The data given in the tables reported in this group of experiments, where the plants were grown to maturity, indicate that boron compounds are toxic in proportion to the boron they carry. These data uphold the conclusions drawn from a comparison of the three boron compounds in the study of the seedling phase of growth of the soybean. On the other hand, Hazelhoff (12) working with corn, beans, and oats, found that injury increased faster with borax than with boric acid. Voelcker (24) has also reported more marked injury with borax than with boric acid.

Description of the visible injury on the leaves

The visible boron injury on the leaves of soybeans is very characteristic and is not easily confused with other forms of toxicity, such as injury due to an excess of iron. It appears that the boron is continually being absorbed, and that very high concentrations cause the plant to turn yellow and die, but that lighter doses concentrate in the rapidly growing cells near the margin of the leaf, and cause their death. The injury may be noted first by the appearance of light colored, partly chlorotic tissue about two to three centimeters on each side of the leaf tip. These affected areas develop a day later into characteristic dark brown spots. An examination of these spots under the microscope shows that the largest number of dark injured cells, which later become transparent, are adjacent to the very small veins. These spots usually originate about one-fourth of a centimeter from the edge of the leaf. (See Plate 1.) They never occur on the cotyledons, as is sometimes the case with other toxic conditions. With further development of the toxicity, spots may appear in the center of the leaf, but they always occur between the larger veins. It was also observed that these spots do not increase to much larger than half a centimeter in diame-

ter, and do not readily cross the larger veins. The dying of the tissue, particularly when the leaves are young, causes the edges to roll either up or down. The injured leaf may otherwise appear to be in a healthy condition until about one-half of the surface area of the leaf becomes affected; the leaf then turns yellow and drops from the plant. This shedding of the leaves, which was very noticeable in Boric Acid Series VII and VIII, probably removes from the plant considerable amounts of the toxic element. Whether this is of any particular value to the plant is questionable. It would appear improbable, however, that once the toxic ion has been fixed in a group of cells in sufficient quantities to produce death it would again become soluble, and therefore toxic

TABLE 8

Average dry weight per plant of soybeans grown to maturity and nutrient solution

CULTURE	BORON PER LITER OF NUTRIENT SOLUTION	*BORIC ACID AVERAGE SERIES VII AND VIII	*BORAX AVERAGE SERIES VII AND VIII
	mgm.	gm.	gm.
1	0	8 5511†	5.7359
2	0.01	9 8400	7.6399
3	0 05	9 3105	6.7854
4	0 10	9 2083	6.5507
5	0.20	11 4770	6 1656
6	0 30	8 8055	7.8172
7	0 40	12 0458	9.0106
8	0.50	11 1057	7.7616
9	0 60	10 6501	7.4355
10	0 70	10.5321	8.8039
11	0.80	8.7565	6.6379
12	0 90	10.5011	5.9596
13	1.00	12 1673	8.6100
14	2.50	12.2756	8.3490

* Borax Series VII and VIII were run at different periods from Boric Acid Series VII and VIII. Average of six plants.

† Average of three plants.

to other cells. Although the injury to the leaves gradually works its way up the plant, it never appears on the young, rapidly growing tissue.

The influence of boron on the development of the root system

A careful examination from time to time of the root systems of all series of plants grown in water culture showed no difference in any of the cultures. This confirmed the results which have been noted previously in the study of the seedling phase of growth, and is directly contrary to the results secured by Warington (25) with the broad bean at the Rothamsted Station.

The influence of boron on the dry weights of plants

The average dry weights of the plants of Boric Acid Series VII and VIII are given in table 8. It was observed, although the figures are not given here,

that only three cultures of the two series had a dry weight at maturity lower than the controls. Certainly the toxic influence of boron at any of the concentrations used was not sufficient to injure the plant materially. In most cultures there was a material increase of dry weight over that of the controls.

The average dry weights of the total plants of Borax Series VII and VIII, which are also given in table 8, show an increase over the checks for the cultures receiving boron. Only one culture of Borax Series VII and VIII showed a dry weight less than the control.

In most of the work previously reported, the dry weights of plants have been taken as the index of toxicity. The results of these experiments indicate that boron is toxic in lower concentrations than is necessary to reduce the dry weight, and that the visible injury to the leaves is by far a superior method of indicating harmful effects on plant growth.

The stimulating influence of boron

The data in table 8 give the first marked indication, so far observed, that boron may be stimulating to plant growth. This stimulation is noticeable in both the boric acid and the borax series. The greatest individual dry weight in the boric acid series was secured with the highest concentration of boron applied (2.5 mgm.) although this plant suffered the greatest from toxicity, as indicated by the visible injury to the leaves. The increase over the controls in the dry weight of all cultures receiving boron appears to be correlated with the increase of boron in the nutrient solutions, but the correlation is not very uniform. This also appeared to be true where borax was applied.

Concentrations of 0.014 to 7.09 mgm. of boron per liter of nutrient solution appeared to be beneficial. It is well to emphasize that in all of the series reported in this paper, cultures containing as low a concentration of boron as 0.01 mgm. per liter of nutrient solution were included. With one exception, all control plants of all cultures appeared to be normal healthy plants, and showed no characteristic dying, as noted at Rothamsted. Of course, it may be that the soybean is not influenced by boron as is the broad bean. Warington (26) did not secure the same results with barley as she did with the broad bean. Agulhon (1) is inclined to believe that boron is "a particular element" and that it may be required by particular groups of plants.

The influence of boron on the shoot-root ratio

Warington (25) found a decided drop in the shoot-root ratio of the broad bean in all cultures where the concentration of boric acid was favorable to plant growth. In those cultures where boron exerted a toxic influence, the ratio rises to about the same level as in the controls. Agulhon (1) has also noted a similar drop in the shoot-root ratio of wheat plants when grown in water culture solutions. Warington (25), however, could find no definite relationship between the shoot-root ratio of variously treated barley plants.

The shoot-root ratios of soybeans grown in the two Boric Acid Series VII and VIII, and of the two Borax Series VII and VIII were determined. It was observed from these data that no definite relationship can be shown between the shoot-root ratios of the variously treated cultures. It thus appears that the root and shoot of the soybean must be affected about equally by the boron compound.

The influence of boron on the absorption of mineral elements

Agulhon (1) and Warington (25) have both found an increase in the green weight value of plants with certain concentrations of boron. Agulhon (1) attributed this to an increase in the water-holding capacity of the plant, resulting from a state of over-mineralization induced by the absorption of boron. It was thought that if this were true, the percentage of ash of the various cultures would show this influence. Ash determinations, therefore, were made of Borax Series IX. These data showed that there is no relationship whatever between the concentration of boron in the nutrient solution and the amount of mineral elements absorbed.

SUMMARY

This piece of research was attempted in order to determine what influence boron exerts upon the growth of soybeans. Experiments were run to determine the influence of boron on the germination of seeds and on the growth of soybeans grown in nutrient solution, sand, and soil. A comparative study was also made of the influence of boron when applied in the form of boric acid, potassium borate, and sodium borate or borax.

A total of 78 germination tests were made. In addition, 136 nutrient solution cultures, 54 sand cultures, and 54 soil cultures were grown. Altogether 732 plants were grown to test out the influence of boron on plant growth. In some of the experiments the plants were harvested when they had reached their flowering stage, whereas in other experiments the plants were grown to maturity before harvesting. Some of the principal results secured from the study are as follows:

Influence on germination

1. The presence of as much as 250 mgm. of boron per liter in the soil (sand) solution entirely prevents the germination of soybean seed.
2. The presence of as much as 10 mgm. of boron per liter in the soil solution is capable of delaying the germination of soybean seed.
3. The presence of as much as 10 mgm. of boron per liter in the soil (sand) solution had such an influence upon germination that chlorotic, or partly chlorotic seedlings were produced.
4. Sassafras loam soil has the power of absorption of boron, and of reducing the toxic action of boron compounds on germination.
5. The influence of boron on germination appears to be identical whether in the form of boric acid, potassium borate, or borax.

Influence of boron compounds on soybean plants grown in sand

1. Visible injury to the leaves appeared on all cultures where as much as 1 pound of borax, or its equivalent, was added per acre.
2. Boric acid, potassium borate, and borax reduced the dry weight of plants when applied in quantities equivalent to 7.5 pounds, or more, of borax per acre. With potassium borate and boric acid this injury was shown when applications of 5 and 1 pounds, respectively, were made.
3. Equivalent amounts of boron in the form of boric acid, potassium borate, or borax, produced about the same visible injury to the leaves.
4. No marked stimulation to growth could be detected where boron compounds were applied to plants growing in sand.

Influence of boron compounds on soybean plants grown in soil (Norfolk sand)

1. Visible injury to the leaves appeared on all cultures where as much as 1 pound of borax, or its equivalent, was added per acre.
2. All boron compounds tested reduced the dry weight of plants where applied in quantities equivalent to 1 pound of borax per acre.
3. Equivalent amounts of boron in the form of boric acid, potassium borate, or borax, produced about the same visible injury to the leaves.
4. No visible stimulation to growth could be detected where boron compounds were applied to plants growing in soil.

Influence of boron on the seedling phase of growth of plants grown in nutrient solution

1. Boron is toxic to soybeans, and this toxicity manifests itself, where small quantities are present, in the form of a characteristic injury to the leaves; or where large quantities are present, in stunting or killing the plant.
2. During the seedling phase of growth of soybeans, at least 0.4 to 0.5 mgm. of boron per liter of nutrient solution is toxic.
3. The toxic influence of boron is practically the same whether it is in the form of boric acid, potassium borate, or borax.
4. Boron is not necessary to the growth of soybean plants during the seedling stage of growth.
5. No marked indication of any stimulating influence of boron could be observed.

Influence of boron on plants grown to maturity in nutrient solution

1. Boron when present in the nutrient solution in concentrations as low as 0.1 to 0.2 mgm. per liter of the solution is able to produce visible toxicity on the leaves of soybeans.
2. The toxicity of boron is practically the same whether it is added in the form of boric acid or borax.
3. Boron is not necessary to the production of mature soybean plants.
4. Boric acid, and to a lesser degree, borax, exerted a stimulating influence on soybean plants, the greatest stimulation appearing when 2.5 mgm. of boron per liter of cultural solution had been used.

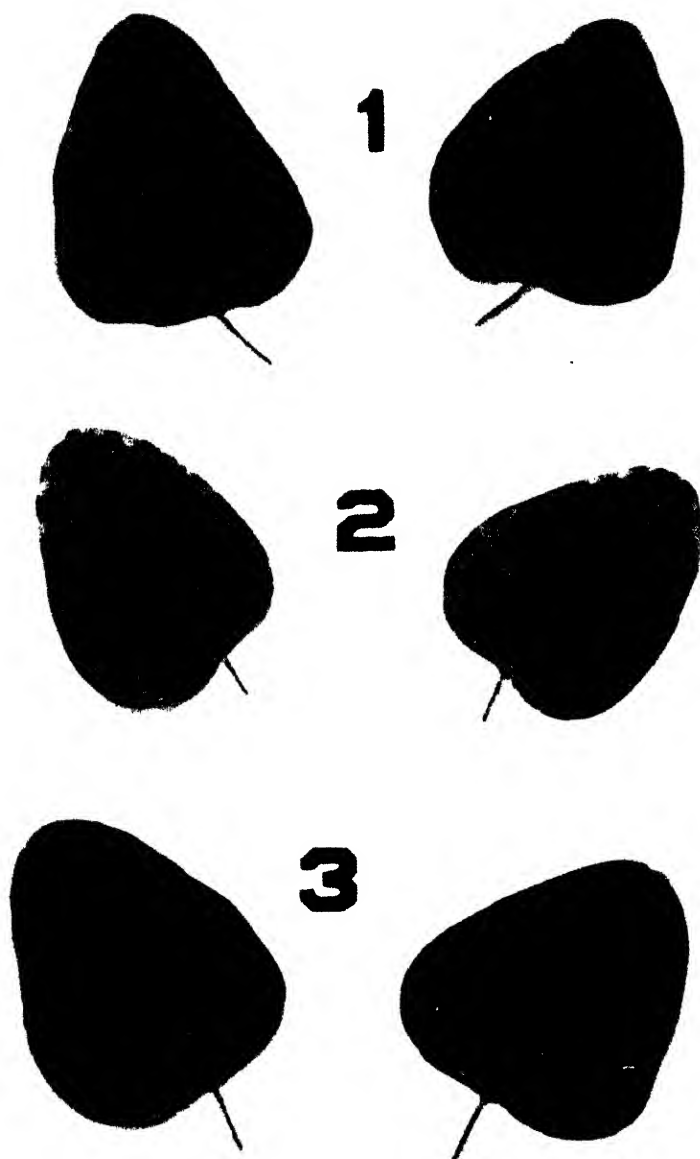
REFERENCES

- (1) AGULHON, H. 1910 Recherches sur la presence et le role du bore chez les vegetaux. Thesis. Paris.
- (2) ALLISON, R. V., AND SHIVE, J. W. 1923 Studies on the relation of areation and continuous renewal of nutrient solution to the growth of soybeans in artificial culture. *Amer. Jour. Bot.* 10: 554-566.
- (3) ARCHANGELI, G. 1885 Sopra l'azione dell'acido borico sul germogliamento dei semi. *Processi verbali Soc. Toscana Sci. Nat*, Pisa 5: 25-29.
- (4) BAUDISH, OSKAR 1923 On the formation of organic compounds from inorganic by the influence of light. *Science* 57 (1477) 451.
- (5) BLACKWELL, C. P., AND COLLINGS, G. H. 1920 Trona Potash—a progress report. S. C. Agr. Exp. Sta. Bul. 202.
- (6) BLAIR, A. W., AND BROWN, B. E. 1921 The influence of fertilizers containing borax on the yield of potatoes and corn, season 1920. *Soil Sci.* 11: 369-376.
- (7) BRECKENBRIDGE, J. E. 1921 Boron in relation to the fertilizer industry. *Jour. Indus. Engin. Chem.* 13. 324-325.
- (8) BRENCHLEY, WINIFRED E. 1914 Inorganic Plant Poisons and Stimulants. Cambridge University Press.
- (9) CALLISON, J. A. 1890 The distribution of boracic acid among plants. *Jour. Analyt. Chem.* 4: 191-197.
- (10) COOK, F. C. 1916 Boron: its absorption and distribution in plants and its effect on growth. *Jour. Agr. Res.* 5. 877-890.
- (11) COOK, F. C., AND WILSON, J. B. 1917 Effect of three annual applications of boron on wheat. *Jour. Agr. Res.* 10: 591-597.
- (12) HAZELHOFF, E. 1913 Ueber die Einwirkung von Borverbindungen auf das Pflanzenwachstum. *Landw. Vers. Sta* 79-80: 399-429.
- (13) HECKEL, E. 1875 De l'action de quelques composés sur la germination des graines (bromure de camphre, borate, silicate et arseniate de soude). *Compt. Rend. Acad. Sci. (Paris)* 80: 1170-1172.
- (14) HOTTER, E. 1890 Ueber das Vorkommen des Bor im Pflanzenreich und dessen physiologische Bedeutung. *Landw. Ver. Sta.* 37: 437-458.
- (15) JONES, L. H., AND SHIVE, J. W. 1923 Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on hydrogen-ion concentration and iron availability. *Ann. Bot.* 37: 355-377.
- (16) KAHLBERG, L., AND TRUE, R. H. 1896 On the toxic action of dissolved salts and their electrolytic dissociation. *Bot. Gaz.* 22: 81-124.
- (17) MAZE, P. 1915 Determination des elements minéraux rares nécessaires au développement du maïs. *Compt. Rend. Acad. Sci. (Paris)* 160: 211-214.
- (18) MOREL, J. 1892 Action de l'acide borique sur la germination. *Compt. Rend. Acad. Sci. (Paris)* 114: 131-133.
- (19) NELLER, J. R., AND MORSE, W. J. 1922 Effects upon the growth of potatoes, corn, and beans resulting from the addition of borax to the fertilizer used. *Soil Sci.* 12: 79-131.
- (20) SCHREINER, O., ET AL. 1920 Crop injury by borax in fertilizers. *U. S. Dept. Agr. Dept. Cir.* 84: 35.
- (21) SHERWIN, M. E. 1920 Effect of fertilizers on germination and seedling growth of corn and cotton. *Jour. Elisha Mitchell Sci. Soc.* 36: 16.
- (22) SKINNER, J. J., AND ALLISON, F. E. 1923 Influence of fertilizers containing borax on the growth and fruiting of cotton. *Jour. Agr. Res.* 23: 433-445.
- (23) TOTTINGHAM, WILLIAM E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133-245.

- (24) VOELCKER, J. A. 1915 The influence of boron compounds on wheat and barley. *Jour. Roy. Agr. Soc. (England)* 76: 347-351.
- (25) WARINGTON, KATHERINE 1923 The effect of boric acid and borax on the broad bean and certain other plants. *Ann. Bot. (London)* 37: 630-672.
- (26) WITTSTEIN, A., AND APOIGER, F. 1857 Entdeckung der Borsäure in Pflanzenreiche. *Ann. Chem. Pharm. (Liebig)* 103: 362-364.

PLATE 1

THE TYPE AND THE EXTENT OF BORON INJURY ON THE FIRST PAIR OF LEAVES OF REPRESENTATIVE PLANTS WHICH RECEIVED EQUIVALENT QUANTITIES OF BORON AS BORIC ACID, POTASSIUM BORATE, AND BORAX
1, Boric acid; 2, Potassium borate; 3, Borax



ORGANIC MATTER CHANGES IN TWO SOIL ZONES, AS INFLUENCED BY DIFFERENCE IN FORM, FINENESS, AND AMOUNT OF CALCIC AND MAGNESIC MATERIALS¹

W. H. MACINTIRE, W. M. SHAW, AND ESTHER M. CRAWFORD

University of Tennessee Agricultural Experiment Station

Received for publication May 15, 1926

It is generally conceded that economic conditions of liming materials are followed by stimulation of bacterial processes. Such processes result in the oxidation of soil organic matter, with the production of nitrates and carbon dioxide. Determinations of residual nitrogen, "humus," and organic CO₂ have therefore been used to measure changes in organic matter. The subject of economic liming and its effect upon conservation of nitrogen and organic matter has been studied in this manner at the Tennessee Station for a number of years. Certain cowpea-wheat rotation experiments (6, 7) and certain cylinder studies (8) that have been reported are being continued. This paper arises from a detached 4-year lysimeter investigation, wherein organic matter changes were incidental to the major objectives of calcium and magnesium outgo, as such outgo was influenced by zone-of-incorporation and by variation in form and amount and degree of fineness of added calcic and magnesian materials.

EXPERIMENTAL

The major variation, that is, zone-of-incorporation, applied in the comparisons among all materials. Hydrated lime at the rate of 2000 pounds of CaO per 2,000,000 pounds of soil, moisture-free basis, was compared with equivalent incorporations of 10-20-, 20-40-, 40-80-, 80-200-mesh and an equal-part composite of limestone and of dolomite, and also with equivalent CaO—MgO incorporations. Three fractional rates of 1000 pounds 500 pounds, and 250 pounds of CaO as Ca(OH)₂, were also included.

The experiment extended over the 4-year period, May, 1921 to May, 1925. The lysimeter equipment has been described, together with soil, method of treatment and materials used (4). Originally the entire mass of screened soil was thoroughly mixed to insure uniform composition. One addition was incorporated in the surface half or zone of each tank of one series, without treatment to the lower zone, whereas in another series each addition was incorporated in the lower zone without any treatment to the upper zone. After

¹ The results were obtained by means of equipment donated by the American Limestone Company and through fellowship assistance provided by the National Lime Association.

4 years' exposure to natural leaching, under fallow and without stirring, the two zones were taken up separately and dried. An entire 2-quart sample

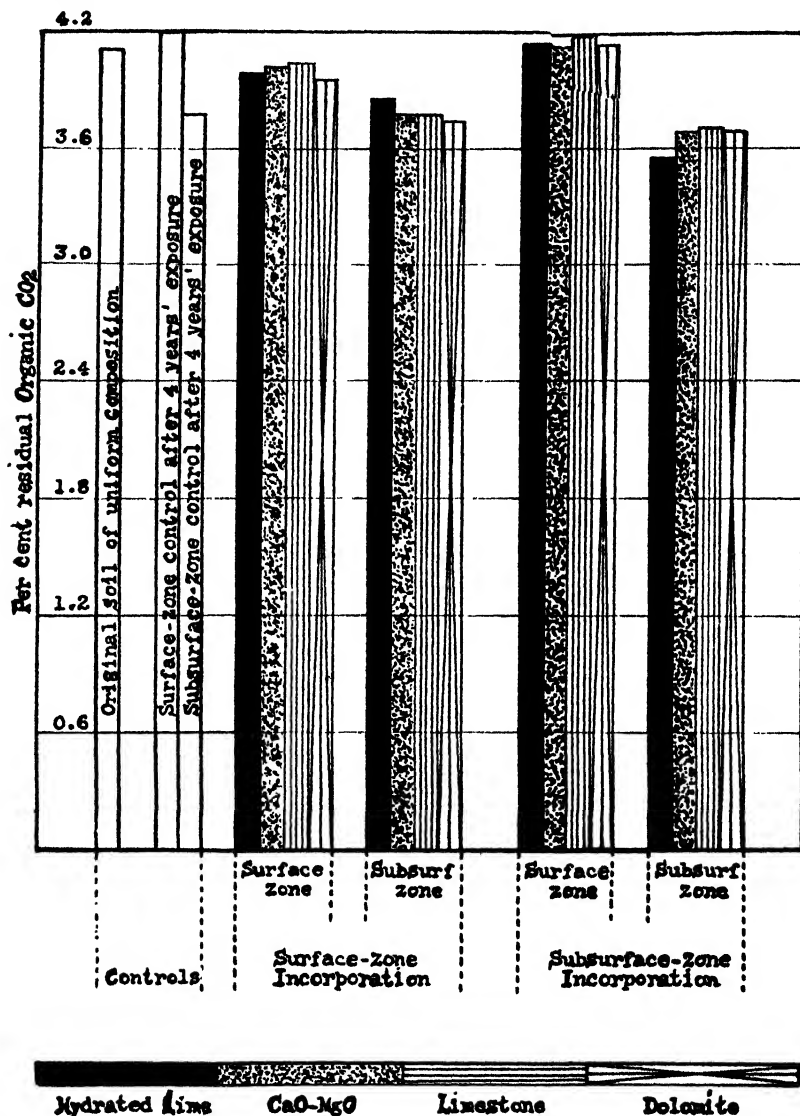


FIG. 1. ORGANIC-CO₂ CHANGES INDUCED IN A LOAM SOIL BY SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF Ca(OH)₂, CaO-MgO, 80-100-MESH LIMESTONE AND DOLOMITE AT THE CONSTANT RATE OF 2000 POUNDS OF CaO PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS, DURING 4 YEARS' FALLOW EXPOSURE WITHOUT STIRRING

from each zone of each tank was ground to pass a 100-mesh sieve and carbonate-CO₂ was determined upon 100-gm. charges (4). Five-gram charges were

burned in an electric furnace, with precautions to preclude vitiation from volatile sulfur, chlorine, and nitrogen compounds. The total combustion- CO_2 determinations were corrected for the previously reported (4) carbonate- CO_2 results.

TABLE 1

Organic- CO_2 content of two soil zones of an originally uniform soil, as influenced by surface-zone incorporations of caustic and natural forms of calcic and magnesian materials and 4 years' exposure in out-door lysimeters

TANK NUMBER	MATERIALS			ORGANIC CO_2						
	Form	Mesh	CaO \approx per 2,000,000 pounds of soil	Surface zone			Subsurface zone			Minus variation of sub- surface zone
				A	B	Aver- age	A	B	Aver- age	
				per cent	per cent	per cent	per cent	per cent	per cent	
80*	None	4 19*	3 81*	0.38
81*	$\text{Ca}(\text{OH})_2$. .	250	4 04	4 04	4 04	3 82	3 83	3 83	0.21
82*	$\text{Ca}(\text{OH})_2$.	500	4 10	4 12	4 11	3.82	3 84	3.83	0.28
83*	$\text{Ca}(\text{OH})_2$.	1,000	4.10	4 16	4 13	3 77	3 79	3 78	0.35
84*	$\text{Ca}(\text{OH})_2$. . .	2,000	3 98	4 00	3 99	3 86	3 86	3 86	0.13
85*	$\text{CaO-MgO}\dagger$.	2,000	4 20	4 20	4 20	3 73	3 74	3.74	0.46
86*	$\text{CaO-MgO}\ddagger$.	2,000	3 87	3 88	3 88	3 82	3 84	3 83	0.05
88*	Limestone	10- 20	2,000	4 05	4 05	4 05	3.87	3 89	3 88	0.17
89*	Limestone	20- 40	2,000	4 01	4 02	4 02	3 82	3 81	3 82	0.20
90*	Limestone	40- 80	2,000	4 05	4 03	4 04	3 83	3 81	3 82	0.22
91*	Limestone	80-200	2,000	4 05	4 04	4 05	3 78	3 80	3 79	0.26
87*	Limestone	Comp	2,000	3 97	3 98	3 98	3 78	3 77	3 78	0.20
93*	Dolomite	10- 20	2,000	4 11	4 11	4 11	3 79	3.76	3 78	0.33
94*	Dolomite	20- 40	2,000	4 04	4 05	4 05	3 88	3 84	3 86	0.19
95*	Dolomite	40- 80	2,000	4.04	4 06	4 05	3 86	3 84	3 85	0.20
96*	Dolomite	80-200	2,000	3 94	3 96	3 95	3 74	3 76	3 75	0.20
92*	Dolomite	Comp.	2,000	4 05	4 06	4 06	3.81	3 82	3 82	0.24
Average						4 05			3 81	0.24

* Average of 6 determinations.

† Burnt dolomite 46.53 per cent CaO and 32.85 per cent MgO.

‡ Mixture of separately calcined oxides.

DISCUSSION

The organic- CO_2 data are given in tables 1 and 2 and condensed in graphic comparisons for the 2000-pound treatments in figure 1.

Before results are discussed, it should be emphasized that the full rates of treatment were incorporated with one-half the entire mass of soil, so that the maximum rate of 2000 pounds CaO-equivalence per 2,000,000 pounds of soil,

or a percentage of 0.1 on the basis of the full depth of soil, was actually a rate of 2000 pounds per 1,000,000 pounds of soil, or a percentage of 0.2 when considered on the basis of direct contact of the additions with one-half of the entire soil mass. In previous studies (3) the same soil received incorporations of burnt and hydrated limes and precipitated CaCO_3 , at the rate of 4000

TABLE 2

Organic- CO_2 content of two soil zones of an originally uniform soil, as influenced by subsurface-zone incorporations of caustic and natural forms of calcic and magnesian materials and 4 year's exposure in out-door lysimeters

TANK NUMBER	MATERIALS			ORGANIC CO_2						
	Form	Mesh	CaO - per 2,000,000 pounds of soil	Surface zone			Sub-surface zone			Minus variation of sub-surface zone
				A	B	Average	A	B	Average	
				per cent	per cent	per cent	per cent	per cent	per cent	
80B	None	4 19*	3 77	3 78	3.78*	0.41
81B	$\text{Ca}(\text{OH})_2$. .	250	4 26	4 23	4 25	3.77	3.78	3 78	0 47
82B	$\text{Ca}(\text{OH})_2$. . .	500	4 20	4 17	4.19	3.73	3 69	3 71	0 48
83B	$\text{Ca}(\text{OH})_2$. .	1,000	4.06	4 10	4 08	3 70	3 72	3.71	0 37
84B	$\text{Ca}(\text{OH})_2$. . .	2,000	4 14	4 13	4.14	3 54	3.58	3.56	0 58
85B	$\text{CaO-MgO}\dagger$. .	2,000	4 09	4 08	4 09	3 62	3 65	3 64	0 45
86B	$\text{CaO-MgO}\dagger$. .	2,000	4 17	4 18	4.18	3 73	3 74	3.74	0 44
88B	Limestone	10- 20	2,000	4 19	4 17	4 18	3 80	3.76	3.78	0 40
89B	Limestone	20- 40	2,000	4 19	4 17	4 18	3 78	3 74	3 76	0 42
90B	Limestone	40- 80	2,000	4 12	4 15	4 14	3.70	3 71	3 71	0 43
91B	Limestone	80-200	2,000	4 25	4 27	4.26	3.70	3 72	3.71	0.55
87B	Limestone	Comp.	2,000	4 18	4 20	4.19	3.72	3.76	3.74	0 45
93B	Dolomite	10- 20	2,000	4 21	4 24	4 23	3 75	3 79	3 77	0.46
94B	Dolomite	20- 40	2,000	4 12	4 08	4 10	3 72	3 74	3 73	0.37
95B	Dolomite	40- 80	2,000	4 07	4 09	4.08	3 68	3 72	3 70	0.38
96B	Dolomite	80-200	2,000	4 12	4.16	4 14	3 69	3 71	3 70	0.44
92B	Dolomite	Comp.	2,000	4 23	4 23	4 23	3.72	3 74	3.73	0.50
Average						4.16			3.72	0.44

* Average of 6 determinations.

† Burnt dolomite 46.53 per cent CaO and 32.85 per cent MgO.

‡ Mixture of separately calcined oxides.

pounds of CaO per 1,500,000 pounds of soil, or a percentage of 0.266. These earlier studies showed that all of the $\text{Ca}(\text{OH})_2$ and CaCO_3 derived from the burnt lime, hydrated lime, or calcium carbonate had disappeared before the end of 10 days. In the present case, it follows, therefore, that any changes induced by the caustic forms for the 4-year period must have come from ab-

sorbed CaO, rather than from any appreciable residues of hydroxide or carbonate forms, except during the first 10-day period. Moreover, the imposition of intensive conditions in the laboratory failed to demonstrate any chemical disintegration of organic matter, so that all decreases found in the present study are considered as the result of biochemical accelerations.

Influence of surface-zone incorporations as measured by the surface-zone controls

It will be noted that the surface-zone of the control gave an organic CO₂ content of 4.19 per cent after 4 years, as compared with 4.12 per cent for a representative composite secured at the time the soil was placed in the tanks. Some oxidation of organic matter must have taken place in the upper zone. Such oxidation was probably at the minimum during the period when the direct rays of the sun were of maximum effect and when the upper surface was a dry crust too low in moisture for optimum bacterial activities. But, an actual *increase* in content over that of the original soil was found and must be accounted for. This may be due to two additive causes working jointly to offset and supplement the loss in original organic matter. The rainfall brings down a considerable amount of soot—as well as dust—which is retained upon, or near, the surface of the soil. No quantitative determinations of soot increment have been made, but observations necessitate consideration of this factor. Some organic matter may have been derived from algae and lichens, in spite of the effort to prevent all plant growth. Gravitational adjustment of soil particles in the unstirred leached soil may also have been a vitiating factor. The final composition of the untreated soil may be considered as having included these factors, as near as it was possible to control them. In the following comparison from the data of table 1, surface zone variations will therefore be dealt with primarily on the basis of the final composition of the upper-zone of the exposed controls, rather than that of the original soil.

Of the 16 treatments 15 caused results lower than the amount found in the exposed, but untreated, control. In the hydrated lime group the 2000-pound incorporation gave the lowest residual and greatest variation from the control. The 250-pound addition came next, with a difference hardly more than that to be permitted for experimental error, whereas the 500-pound and 1000-pound additions were practically identical, with some indication of decrease below the content of the exposed control.

The burned dolomite treatment was the one exception which showed no difference from the control, but the corresponding oxide mixture showed a considerable decrease, one somewhat greater than that found for the chemically equivalent hydrated lime treatment.

Each of the limestone separates and the composite showed a diminished organic matter content. The average reduction for the group was 0.16 per cent. The differences between the several limestone separates were small, however, and no definite influence of degree of fineness was registered. In

general the same obtains for the dolomite group, although the inactivity of the 10-20-mesh separate is definitely established as compared with that of the 80-200-mesh separate. The 5-unit average for limestone is practically identical with that for dolomite. There were found, therefore, no outstanding differences which could be attributed to variation in degree of fineness, nor to differential influences exerted by surface-zone incorporations of the two types of limestone.

Influence of surface-zone incorporations upon subsurface-zone changes

The data of table 1 also show that, for every treatment, the subsurface-zone content was less than that of its respective overlying treated zone, but the same variation was found also for the controls. The subsurface-zone of the exposed untreated tank shows an organic matter content 0.38 per cent less than that of its overlying zone and 0.31 per cent less than the original soil of initial uniform composition. This disparity was larger than that found in any of the 16 treatments, with one exception. As a whole the entire 16 treated tanks show a marked uniformity of lower-zone content and their average is identical with the residual content of the untreated control. It thus appeared that the amounts of alkali-earths which have passed from the upper zone and which have been fixed in the lower zone have not induced acceleration of biochemical processes in the lower zone.

The moisture content of the lower zone is nearly always greater than that of the upper-zone during the seasons when bacterial activities are most pronounced. Moreover, the lower-zone was never subject to the direct rays of the sun during the 4-year period. Brown (1) and King and Doryland (2) have shown that bacteria may be expected to be more abundant and physiologically more active in the second 4 inches of the surface soil. These factors are most probably responsible for the greater oxidation shown as a zone influence, without additive treatment.

Influence of subsurface-zone incorporations upon surface-zone changes

The data of table 2 show that in 4 of the 16 tanks, which received subsurface-zone treatment but no additions to the upper-zone, the organic matter content of the upper zone at the end of 4 years was slightly greater than that of the upper-zone of the exposed no-treatment tanks. However, 12 of the tanks showed a gain over the content of the original soil. In 5 of the 12 cases which showed an amount less than the exposed control, there was an analytical variation of only 0.01 per cent, whereas in the remaining 7 tanks the maximum disparity was 0.11 per cent. No definite variation therefore appeared to justify the assumption that underplaced caustic and carbonate treatments produced differential effects upon the overlying untreated zone. The average of the 3 fractional treatments of hydrated lime—250 pounds, 500 pounds, and 1000 pounds—the three 2000-pound caustic treatments, and the 10 limestone and

dolomite treatments shows a 4.16 per cent content against 4.19 per cent for the soil which received no treatment in either zone. It is thus apparent that no ultimate decrease in organic CO₂ was produced in the upper zone as a result of the lower-zone incorporations.

Influence of subsurface-zone incorporations as measured by the subsurface-zone controls

The final organic matter content of the lower zone of the control was identical with that of each of the tanks which received lower-zone incorporations of hydrated lime at the 250-pound rate and limestone and dolomite of 10–20-mesh fineness. The 500-pound and 1000-pound rates of hydrated lime showed an identical disparity of only 0.07 per cent, whereas the maximum minus variation induced by any one of the 8 limestone and dolomite separates and by the CaO-MgO mixture was 0.08 per cent. The burned dolomite and CaO-MgO mixture registered 0.14 per cent and 0.04 per cent, respectively, less than the control, whereas the maximum decrease of 0.22 came from a chemically equivalent addition of hydrated lime. It has been pointed out that this hydrated lime incorporation was fixed by the soil in less than 10 days, and that only the finer limestone separates had been completely disintegrated (4) after 4 years, so that the somewhat greater decomposition must be attributed to initial influences resultant from greater availability and more rapid assimilation. In other words, practically the full amount of the hydrated lime incorporation was quickly “fixed” (5) and effective throughout the entire 4-year period, whereas the limestone incorporations were not; varying fractions of the several limestone incorporations were, in a sense, inert during their persistence as undistintegrated particles. The average of all 16 additions, 13 of which were at the same rate, amounted to 3.72 per cent, as compared to 3.78 per cent where no addition was made to either zone.

Comparison of all surface-zone units, with and without incorporations

In table 1 the changes induced by the 16 surface-zone incorporations are given, whereas table 2 gives the same number of units which were subject to no alkali-earth increments, except any which might have moved upward from the lower zone of incorporation. It has been shown that surface-zone additions induced no decrease in subsurface-zone organic matter and that subsurface-zone incorporations failed to decrease the organic matter content of the untreated upper zone. Comparisons may therefore be made between the 16-unit series to which additions were made in the upper zone and the corresponding series where no surface-zone incorporations were made. These respective comparisons are given in condensed form for the 2000-pound CaO-equivalent incorporations in figure 1. The more extensively decomposed and “fixed” (5) 80–200-mesh limestone and dolomite separates are utilized. In the case of the no-treatment surface group, 87B-91B, the closely agreeing units were

averaged. When the results from the surface-zone, to which the different additions were made, are compared with the results from the same zone without additions, some stimulation in oxidation of soil organic matter was registered by 14 of the 16 treatments, if it may be assumed that the same increment of organic matter came to each tank from rainfall and restricted growth of the lower forms of plant life. The reverse obtained, though to an extent of only 0.05 per cent, for the 1000-pound CaO-equivalence of hydrated lime and also, to the extent of 0.11 per cent, for the burnt dolomite addition. As an average of all fractional and full treatments, the alkali-earth additions gave organic CO₂ residuals 0.13 per cent less than the average given by the 16 tanks where the surface-zone was untreated. The thirteen 2000-pound surface-zone incorporations gave fairly uniform residuals. The same holds for the 13 corresponding tanks where no incorporations were made in the upper-zone. If the difference found between their respective averages of 4.03 per cent and 4.16 per cent shows an actual loss of 0.13 per cent for the 4-year period, or an annual loss of 0.0325 per cent, such a small loss would probably be offset by organic matter derived from crop residues.

Comparison of all subsurface-zone units, with and without incorporations

The foregoing basis of comparison may also be applied to the lower zone. Each addition gave an apparent enhancement in oxidation of the lower-zone organic matter. In some cases the enhancement is relatively small. The minimum for the limestone series was 0.04 per cent and the maximum 0.11 per cent for the composite and 40–80-mesh product, respectively. Corresponding extremes of 0.01 per cent and 0.15 per cent obtained for the 10–20-mesh and 40–80-mesh separates of dolomite. No positive relation between rate of treatment and increase in oxidation showed for the 250-, 500-, and 1000-pound CaO additions, whereas the two CaO-MgO supplements were practically identical. The greatest enhancement of all came from the 2000-pound CaO-equivalent incorporation of hydrated lime, where the increased oxidation in the lower-zone over that of the upper-zone amounted to 0.30 per cent. This represents practically the same effect as that registered by the hydrated lime in the upper zone. The grand average of the 16 subsurface-zone residues, where no additions were made to the lower zone, was 3.81 per cent against 3.72 per cent as a corresponding average from the 16 subsurface-zone incorporations, i.e., an average increase of 0.09 per cent in oxidation as a result of additions. A similar comparison of averages for the thirteen 2000-pound incorporations gives an increase of 0.10 per cent attributable to the average accelerative effect from the subsurface-zone incorporations.

Zone effect versus influence of additions

From the comparison of surface-zone incorporations with the untreated exposed control, it appeared that 15 of the 16 treatments caused some enhance-

ment in oxidation of organic matter; but from an inter-zone comparison for each of these tanks, it developed that the subsurface-zone conditions minus additions were more conducive to oxidation than were the surface conditions plus treatment. The average for the subsurface-zone-condition effect without incorporations over that of the surface-zone effect plus treatments amounted to 0.24 per cent. This constitutes an absolute zone-basis loss of 2400 pounds or 4800 pounds upon the full 2,000,000-pound basis. It has been pointed out that this greater loss in the lower zone is not to be attributed to any acceleration induced by influx of alkali-earths from the several treatments in the upper zone, since the disparity between the final contents of the two zones in the two untreated exposed controls was greater than analogous disparities in 15 of the 16 incorporations.

From the data of table 2 it appears that the combined factors of alkali-earth treatment and more favorable conditions for bacterial activities in the lower-zone were responsible for an average disparity of 0.44 per cent in comparison with the average of all exposed no-treatment upper-zone units. This disparity, however, represents the differential from a grand no-treatment surface-zone average which showed very little deviation from the organic- CO_2 content of the exposed control. In the several individual disparities used to obtain this average, each minus variation was larger than its corresponding one in table 1. It has been pointed out that for any oxidation which occurred in the surface zone there was an apparent offset from organic matter increments, or gravitational adjustment.

The maximum differentials between subsurface-zone and surface-zone activities came from the 2000-pound hydrated lime treatment and from the 80–200-mesh limestone separate. The other treatments do not show sufficiently great nor consistent differences to warrant special stress.

The average effect of the thirteen 2000-pound surface-zone incorporations was to produce an oxidation 0.14 per cent in excess of that which took place in the untreated soil, so that the full average oxidative increase exerted in the lower zone, per se and minus contact treatments, was therefore found to be 0.38 by the two calculations $4.19 - 4.05 + 0.24 = 0.38$ and $4.19 - 3.81 = 0.38$. Similar calculations of $4.19 - 4.16 + 0.44 = 0.47$ per cent and $4.19 - 3.72 = 0.47$, give the greater average oxidation exerted by the lower zone plus contact treatments. An average value of 0.09 per cent is thus obtained as representing the accelerative oxidation exerted by a 2000-pound CaO -equivalent during the 4-year period. Again, from the 4.03 per cent average for the thirteen 2000-pound CaO -equivalent incorporations in the surface-zone and the corresponding 4.16 per cent surface-zone average in the series which contained no surface-zone incorporations, it appears that the lime incorporations produced an average organic CO_2 decrease of 0.13 per cent in the surface zone during the 4-year period. As a general conclusion, it may be said that an average annual decrease of from 0.025 to 0.03 per cent organic CO_2 occurred as a result of the lime additions, irrespective of zone of incorpora-

tion. This may be considered as fairly representative of the value of all 2000-pound incorporations, with the exception of the hydrated lime to which a higher value should be given.

As a salient point, it may be stressed that the zone influence effect upon biological conditions appears to have been a more potent factor than ameliorants, for this particular soil.

SUMMARY

Determinations of organic matter changes in surface-zone and subsurface-zone samples from 34 lysimeters are given to show the influence of surface-zone and subsurface-zone incorporations of 2000-pound and fractional incorporations of hydrated lime and 2000-pound CaO-equivalences of burnt dolomite, separately calcined mixtures of CaO and MgO, 4 separates and their equal-part composite of limestone and of dolomite after a period of 4 years' exposure without crops and without stirring.

The final organic CO₂ content of the surface-zone of the untreated exposed soil was somewhat greater than that of the original soil, whereas the subsurface-zone content was decidedly less. This offset to surface-zone oxidation was attributed to the combined agencies of soot depositions, and algae and similar growth upon the upper crust and to possible gravitational readjustment of soil particles. The marked loss was attributed to greater bacterial activities induced by the more moist conditions of the lower zone, during the period of maximum bacterial activity, and by the absence of any direct sunlight.

Of the 16 surface-zone incorporations, 15 showed oxidation of organic matter, on the basis of the content of the exposed untreated control. The decreases induced by the 4 rates of hydrated lime were not extensive, nor in proportion to the rate, although the maximum rate gave the maximum decrease.

No positive differences in organic-CO₂ residues were found to be attributable to variation in fineness of limestone in the surface-zone, and although some variation was noted between the coarsest and finest of the dolomite separates, the group averages for the two limestones were identical.

All untreated subsurface-zones were found to be of lower organic matter content than their corresponding overlying treated zones, although in 15 of the 16 treatments the differences were less than the zone variation found in the controls. This uniformity in lower-zone composition shows that no appreciable activation was induced by calcium-magnesium leachings from the incorporations made to the upper zone.

No evidence was adduced to show that subsurface-zone incorporations had any accelerative effect upon oxidation of surface-zone organic matter.

With the exception of two of the 2000-pound caustic additions, no marked variation from the control was obtained, nor was any influence of fineness shown for lower-zone incorporations.

Of the 16 surface-zone incorporations, 14 gave organic matter residues which

were less than their respective surface-zone analogues in which no incorporations were made.

Each of 15 of the 16 subsurface-zone incorporations resulted in a decrease in organic matter beyond that of its corresponding subsurface-zone where no incorporation was made, the maximum variations having been produced by the 2000-pound rate of hydrated lime and its equivalent of 80-200-mesh limestone.

In general, the accelerative effects of the liming materials were comparable in the two zones.

The zone-influence appeared to be more potent than that of alkali-earth incorporations in producing oxidation of the organic matter of this particular soil under fallow conditions without stirring and with only natural rain water.

REFERENCES

- (1) BROWN, P. E. 1912 Bacteria at different depths in some typical Iowa soils. *Iowa Agr. Exp. Sta. Res. Bul.* 8
- (2) KING, W. E., AND DORYLAND, J. T. 1909 The influence of depth of cultivation upon soil bacteria and their activities. *Kans Agr. Exp. Sta. Bul.* 161: 213-215.
- (3) MACINTIRE, W. H. 1919 The carbonation of burnt lime in soils. *Soil Sci.* 7: 325-453.
- (4) MACINTIRE, W. H., AND SHAW, W. M. 1925 The disintegration of limestone and dolomite separates as influenced by zone of incorporation. *Soil Sci.* 20: 403-418.
- (5) MACINTIRE, W. H., AND SHAW, W. M. 1926 Fixation of calcium-magnesium from burnt limes, limestone and dolomite incorporations in two soil zones. *Soil Sci.* 22: 109-122.
- (6) MOOERS, C. A. 1926 Effects of liming and green-manuring on soil supplies of nitrogen and humus—Results of 20-years experiments in a cowpea-wheat rotation. *Univ. Tenn. Agr. Exp. Sta. Bul.* 135.
- (7) MOOERS, C. A., HAMPTON, H. H., AND HUNTER, W. K. 1912 Fertility experiments in a rotation of cowpeas and wheat: Part III. The effect of liming and of green manuring on the soil content of nitrogen and humus. *Univ. Tenn. Agr. Exp. Sta. Bul.* 96.
- (8) MOOERS, C. A., AND MACINTIRE, W. H. 1921 The comparative effects of various forms of lime on the nitrogen content of the soil. *Jour. Amer. Soc. Agron.* 13: 185.

THE PHENOMENA OF CONTRACTION AND EXPANSION OF SOILS WHEN WETTED WITH WATER

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication June 1, 1926

Soil colloids swell when they take up water. The question is, however, does the total or absolute volume of the colloids and water considered together, increase or decrease when the dry colloids are brought in contact with water. If the absolute volume increases, it indicates that the swelling is greater than the volume of water taken up in the process; if the absolute volume decreases, it indicates that some of the water adsorbed in the swelling process has been compressed; if, on the other hand, the absolute volume remains unchanged, it would logically seem that the swelling of the colloids is equal to the volume of water taken up, and that the colloid has not expanded additionally, nor has the water contracted or been compressed. It is the object of this paper to present experimental data bearing upon these points.

PROCEDURE AND METHODS

Three different and distinct methods were tried in measuring the volume changes that take place when dry soils or colloids, are brought into contact with water. The first method consisted of rolling puddled soil into certain size pellets, drying them, then dipping them a few times in gelatine solution until a good coating of gelatine was formed around their surface and drying them again either in room temperature or in an oven at 110°C. temperature. These pellets were then dropped into a dilatometer containing water, which was maintained at a definite temperature in a bath, usually at 20°C. This dilatometer was quickly stoppered and placed back in the constant temperature bath and the readings on the dilatometer stem were taken regularly until they became constant, which indicates that the dilatometer contents have attained the temperature of the bath. Inasmuch as the dry gelatine does not dissolve at 20°C. and the water could not penetrate it in the short time required for the attainment of equilibrium, the soil in these pellets remained dry. In order that the soil might come in contact with the water, the dilatometer with its contents was placed in a water bath having a temperature of about 35°C., and kept there for a few minutes until the gelatine coating began to dissolve and the water to penetrate the soil. The dilatometer was then brought back to the original temperature, allowed to attain equilibrium with the original temperature, and the readings were taken again. With the knowledge of the

readings before the dilatometer was placed at the higher temperature bath and after it was brought back to the original temperature bath and equilibrium attained, any differences could be easily ascertained. Inasmuch as the gelatine

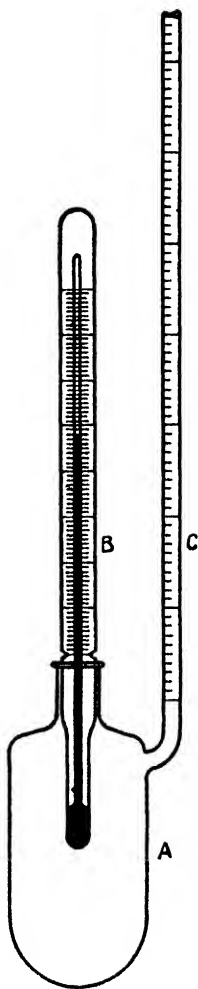


FIG. 1. DILATOMETER FOR MEASURING VOLUME CONTRACTION OF SOILS WHEN WETTED WITH WATER

also takes up water, allowance for this error was made in the final calculations of the results.

The second method consisted of placing a certain quantity of dry soil in a dilatometer, covering it with a layer of dry mercury, then filling the remainder of the dilatometer with water and stoppering it. The mercury being heavier than water, prevented the water from coming in contact with the soil until the

desired time. This dilatometer was then placed in a constant temperature bath until equilibrium was reached, and the reading on the stem was recorded. Then the dilatometer was tilted, allowing the water to come in contact with the soil. After the soil was thoroughly wetted, the apparatus was allowed again to come to equilibrium in the constant temperature bath and the final reading again recorded. Provided no errors entered in the experiment, any differences in the two readings should show contraction or expansion in the absolute volume, due to wetting of the soil colloids with water.

Although both of the foregoing methods gave results which were interesting and significant and indicated that a change did take place in the volume when soils and water were brought in contact with each other, yet these results did not possess as great accuracy as was desired. One of the features which seemed to introduce a considerable error in the results was the air contained in the soil. On account of its compressibility and certain other properties, the air enclosed either in the pellets or under the mercury layer seemed to introduce a definite and variable error.

To overcome this difficulty, it was decided to devise a new method, wherein the factor of soil air was entirely eliminated. Accordingly, the next or third method that was employed consisted of placing a definite quantity of soil in the dilatometer, drying it in an oven at 110°C . for 24 hours, then quickly but gently pouring into the dilatometer sufficient carbon tetrachloride to cover the soil. Then with a glass rod the soil mass was stirred and the air was all displaced by the organic liquid. The dilatometer was then filled with water, stoppered, and placed in a constant temperature bath, and after equilibrium was attained, it was tilted allowing the water and the soil to come into intimate contact. The readings before and after the soil and water were mixed showed the differences, if any, that occurred in the volume.

This method worked remarkably well. As carbon tetrachloride is heavier than water, the latter could be prevented from coming into contact with the soil before the desired time; carbon tetrachloride penetrates the soil mass sufficiently to displace completely all the air present, and the water in turn displaces this carbon tetrachloride so that the reaction taking place between the soil and the water is the same as though the soil were never treated with carbon tetrachloride or as if this liquid were not present. In view of all these facts, the method is exceedingly accurate, simple, and practical, and is probably most exceptional for investigations of the type herein described.

It might be argued that if the carbon tetrachloride wets the soil so as to displace the air from its pores and from around its particles, the water probably cannot wet the soil completely and consequently probably there cannot be the same kind of reaction as if the organic liquid were not present. However, experiments conducted during this investigation as well as those reported in previous publications (1) show that the presence of the carbon tetrachloride has absolutely no adverse effect on the reaction between the soil and water, especially in the case of the mineral soils. For instance, if water is added to

soils, except muck and peats, in the dry condition, while they are immersed in carbon tetrachloride or ligroin, the amount of heat evolved is practically the same as in water alone. This most interesting and significant fact indicates that the specific attraction of the solid materials for water is not destroyed by the presence of carbon tetrachloride or ligroin; and that this attraction will penetrate a solid film of carbon tetrachloride or ligroin to reach the water and

TABLE I

Relationship between volume of contraction when soils are wetted with water and their respective colloidal content and heat of wetting values

SOIL	VOLUME OF CON- TRACTION PER 100 CM	COLLOIDAL CONTENT	HEAT OF WETTING PER GRAM	COLLOIDAL VOLUME OF CONTRAC- TION	HEAT VOLUME OF CONTRAC- TION
	cc.	per cent	calories		
Sand	0 033	1	0 350	30 30	5.30
Fresno Sand Loam	0 216	18 00	1 120	83 30	5.18
Hillsdale Sand Loam A ₂	0 206	15 73	0.930	76 35	4 51
Hillsdale Sand Loam B ₁	0 240	18 70	1 190	77 50	4 95
Hillsdale Sand Loam B ₂	0 233	20 42	1 240	87 60	5 32
Napanec Silt Loam A ₁	0 550	35 57	1 960	64 67	3 56
Napanec Silt Loam C	0 337	50 76	1 990	150 60	5 90
Mich. Silt Loam	0 593	39 04	3 600	65 80	6 58
Ohio Silt Loam	0.433	35 00	2 109	80 80	4.87
Tenn. Silt Loam.	0 616	32 41	2.450	52.61	3.97
Minn. Carrington Silt Loam.	2 533	56 44	9.340	22 28	3.69
Ill. Bloomington Clay Loam.	1 633	40 00	4.460	24 49	2 73
Ill. Urbana Clay Loam	1 570	41 95	3 640	26 71	2 31
Calif. Yolo Loam	1 066	48 49	4.490	45 48	4.21
Calif. Yolo Clay Loam	1 350	59 54	5 930	44.10	4 39
Calif. Yolo Clay.	1 330	58 34	6.430	43 89	4.83
Ontonagon Silt Loam A ₁	0.850	60 65	3.940	71.35	4 63
Ontonagon Silt Loam A ₂	0 940	57.60	4.230	61.27	4 50
Ontonagon Clay B ₁	0 9033	70 00	6 600	77.49	7.30
Colloids from Ontonagon Silt Loam A ₁	1 733	100 00	9 600	57.70	5.53
Colloids from Ontonagon Silt Loam A ₂	1 430	100 00	9.660	69.93	6 75
Colloids from Ontonagon Clay B ₁	1 533	100 00	10.420	65.23	6.79
Muck	4.666		30.100		6.45
Silica Gel	1.620		24.500		
Fullers earth.	4 266		16.000		
Ontonagon Clay B ₁ (moist)	0	70.0			

thus satisfy itself. The distance to which this force will be felt, even through an intervening solid film of another liquid, is considerable.

Hence any objection that might be raised against the use of carbon tetrachloride in the present experiments seem to be set aside by actual tests.

The dilatometer apparatus used is shown in figure 1. It consists of two parts, the bulb and the stem. The bulb has a capacity of 100 cc. The stem

is a 2-cc. pipette which is calibrated to 0.01 cc. and the readings can be estimated to a smaller value than that. The mouth of the bulb was stoppered with cork or rubber, both of which proved satisfactory.

EXPERIMENTAL DATA

In table 1 are presented the experimental data obtained on the volume changes when soils dried at 110°C. for 24 hours, are brought in contact with water, by the third method. The amount of soil used in all cases, was 30 gm., calculated on the absolute dry basis. The figures presented herewith, however, are calculated on the basis of 100 gm. of soil. The figures show the amount of contraction, in cubic centimeter that the original volume underwent, when the water and the soil were brought into intimate contact, or when the soil was perfectly wetted and saturated with water.

An examination of the data in the above table reveals immediately many most interesting and significant facts. In the first place it shows that when oven-dry soils are wetted with water a contraction takes place. The volume of this contraction varies markedly with the different soils—0.033 cc. per 100 gm. of soil in the case of sand, 0.616 cc. in Tennessee silt loam, 1.350 cc. in Yolo clay loam, 2.533 cc. in Carrington silt loam, and 4.666 cc. in muck. Apparently, the soils that tend to give the smallest value of contraction are the light soils, especially the sand; and the soils that tend to give the highest value of contraction are the heavier or colloidal types of soil and the organic soils. Of all the materials used, muck gave the highest value, with Fullers earth coming next in order. From the results with muck, it would be logical to conclude that soils containing considerable amounts of organic matter would yield a correspondingly larger value of contraction, and such appears to be the case as evidenced by the results with Minnesota Carrington silt loam, and with Illinois Bloomington clay loam. The interesting thing to be noticed is that mineral soils with organic matter give as great or greater values of contraction as pure colloids extracted from mineral soils. For instance, the colloids extracted from the A₁, A₂, and B₁ horizons of the Ontonagon soil, which is classed as silt loam in the first two horizons and as clay in the last, do not show any greater volume of contraction than do some of the soils containing various amounts of organic matter. On the other hand, the colloids gave almost twice as great volume of contraction as the corresponding soils from which they were extracted. In this connection it is of much interest to ascertain what relationship there really is between the volume of contraction, colloidal content, and heat of wetting of the different materials used. This relationship is represented in table 1, which contains the values for contraction colloidal content as determined by the heat of wetting method, and by the heat of wetting values.

By comparing first the colloidal content with the volume contraction in table 1 it is seen that there is a general relationship, but not always a very close one, between them. There is a general tendency for soils with the largest amount of colloidal material to give the greatest amount of volume contraction,

but there are some exceptions. For instance, the A_1 horizon of Napanee silt loam has 35.51 per cent of colloidal material and shows a volume contraction of 0.550 cc., whereas the C horizon of the same soil has 50.76 per cent of colloidal material and shows only 0.337 cc. of volume contraction. There is, of course, a reason for this discrepancy. The C horizon contains a very high content of carbonates and has not undergone the extreme weathering process that the above horizons have. Furthermore, the A_1 horizon of this soil contains some organic matter which tends to destroy any close relationship. Possibly the two factors that tend to prevent a very close relationship between the volume of contraction and colloidal content, are differences in organic matter content and in activity or state of hydration of the materials.

By considering next the volume contraction with the heat of wetting, it will be readily seen that there is a rather close relationship between them. Indeed, the ratio ranges from 2.31 to 7.30 between the mineral soils and from 2.31 to 6.45 between the lowest value in the mineral soils and muck. Apparently, this is a little closer relationship than that between the volume contraction and the colloidal content.

The question now is, what causes the contraction in volume when absolutely dry soils are wetted with water.

A comprehensive consideration indicates that the volume contraction in the case of the soils, is due to two chief factors (a) chemical hydration of water and (b) physical compression of water. Of the two, the last is probably of the greatest importance. In both cases, however, there is a condensation of water and consequently a decrease in the original volume.

One of the strongest evidences that the volume contraction is due to the above factors, is the heat evolved when dry soils are wetted with water. The evolution of heat of wetting represents an expenditure of energy on either one or both of the reacting materials. Since the soil colloids, which are mainly responsible for the heat of wetting, are comparatively insoluble, practically the entire expenditure of energy must be at the expense of the water alone. This expenditure of energy on the part of the water also represents a transformation in its state of aggregation, probably from the liquid to the solid or semi-solid phase. In the case of solid materials, such as soil colloids, which adsorb water as film on their surface, the film must be under great compression, and probably approaches the solid state of aggregation. The force with which the effective water film is adsorbed and probably compressed, amounts to more than 150,000 atmospheres per 100 gm. of soil, as shown in previous investigations (2). This water thus compressed must be represented, therefore, in the volume contraction under consideration.

But how is the general notion explained that soil colloids swell when they take up water, if there is an actual decrease in the original total volume? The general notion takes into consideration the volume of the soil alone and not that of the total volume of the soil and water together. It is true that the apparent volume of soil colloids increases when they absorb water, but this

increase or expansion is due to the water they take up or imbibe. The greater the amount of water the material will imbibe, the greater its expansion should be. In this connection it is interesting to observe that of all the soil constituents, the organic matter undergoes the greatest swelling when it takes up water. Yet it has been seen in the present investigation that muck and soils containing organic matter caused the greatest amount of contraction of the absolute original volume. It would appear very suggestive, therefore, that there might be a connection between the amount of water that is compressed and the apparent swelling of the material.

A few observations made during the course of the investigation might be mentioned here. If the soil contained its normal volume of air, there would always be an increase instead of a decrease in the volume when the soil was wetted with water. The increase in volume would vary with the amount of air present. When the air, however, was displaced, as was done by carbon tetrachloride, then the volume always decreased and the results could be repeated almost exactly. Another fact observed was that when the soils were brought into contact with water after being immersed under the column of carbon tetrachloride, the mineral soils would wet with water almost instantaneously, but the mucks, peats, and soils containing high organic matter would wet very slowly—a complete wetting would finally take place, such as in the mineral soils, but it would take longer. The length of time, however, was considerably shortened by shaking the contents.

SUMMARY

An investigation was conducted to ascertain the volume changes that take place when absolutely dry soils are wetted with water.

A specially constructed dilatometer was employed for measuring these volume changes.

It was found that when absolutely dry soils are wetted with water, the original volume of the soil and water is decreased. This volume contraction varies with the different soils. The organic matter constituent tended to give the greatest volume contraction.

An examination was made to ascertain what relationship this volume contraction has to the colloidal content, to the organic matter content, and to the heat of wetting phenomenon. The results showed that the volume contraction has a very close relationship to the organic matter content and to the heat of wetting phenomenon. It also has a relationship to the colloidal content, but this is not so consistent and so close as in the other two cases.

Although soil colloids expand when they take up water, the total volume of the colloids and water considered together, undergoes a contraction.

This contraction in the volume is attributed principally to the condensation of some of the water on the surface of the soil particles or colloids. A small portion may also be due to hydration.

REFERENCES

- (1) Bouyoucos, G. J. 1918 Relationship between the unfree water and the heat of wetting of soils and its significance. Mich. Agr. Exp. Sta. Tech. Bul. 42.
- (2) Bouyoucos, G. J. 1925 Heat of wetting of soils dried at different temperatures and the force at which soils adsorb water. *Soil. Sci.* 20: 67-72.

COLLOIDAL BEHAVIOR OF SOILS AND SOIL FERTILITY: III. CATION REPLACEMENT AND SATURATION OF SOIL WITH Ca¹

J. S. JOFFE AND H. C. McLEAN

New Jersey Agricultural Experiment Stations

Received for publication May 24, 1926

The response of some soils to lime has been known and liming for soil improvement has been practiced for a long time. Although the Ca ion is one of the essential plant constituents, lime has not been applied as its source. Until quite recently the sole object in liming soils has been to neutralize the acids in the soils; the real function of lime, notwithstanding the voluminous researches, has long remained obscure. Only after the factors involved in the process of base exchange were determined and after the lime problem was attacked from the physico-chemical standpoint has the solution been approached.

It has been pointed out (5) that, from the standpoint of crop producing power, soil constituents may be grouped into two broad divisions: active and passive. The passive consists of that portion of the soil which is practically inert as far as the immediate fertility of the soil is concerned: it serves the purpose of mechanical support. In the process of weathering, some of the representative classes of this passive division, like the feldspars, slowly disintegrate from the surface. The disintegration products replenish the active division in which the life of the soil is concentrated. It is this active division that comprises what is known as the soil "complex capable of base exchange." The hydrated aluminum silicates with replaceable Ca, Na, K, and sometimes Mg and H are representatives of this complex. To this portion belongs also that class of humus compounds which are capable of forming complex humates of Ca, Mg, Na, K, or H. It is within this division of the soil make-up that most of the reactions in relation to release and retention of plant-food take place. This active portion is chiefly of colloidal nature and is therefore sometimes referred to as the "colloidal fraction" of the soil.

Capacity for base exchange is an inherent property of every soil and is practically constant unless the soil undergoes drastic treatments that cause deterioration, as shown below. Whenever a soil undergoes cropping, with or without fertilization, the qualitative relationships, but not the capacity, of the various cations in the soil complex capable of exchange varies. If hydrogen ions predominate in the complex, the soil becomes unsaturated and

¹ Paper No. 292 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

acidity appears. This condition is accompanied by a diminution of the other cations, such as Ca, Mg, K, and Na, which are forced out by the H ions (5).

The acid reaction is thus responsible not only for the acid condition per se, which in turn results in solubilizing the harmful Fe and Al ions, but also for depleting the soil of its valuable bases essential for plant growth. It is also important to remember that a complete unsaturation is inductive to a loss of the complex capable of base exchange, both mineral and organic. It has been pointed out (5) that the so-called zeolites with hydrogen as the only cation are more soluble than those with bivalent cations, or with a combination of mono- and bivalent cations, and under certain conditions may be leached out. The same thing occurs with the humates when their cations are replaced with hydrogen.

In order to determine the capacity of any soil for base exchange, 10-gm. samples are treated with 250 cc. of neutral *N* NH_4Cl , left in contact with it for 6 days, and filtered. The soil on the filter is then washed with fresh portions of NH_4Cl solution until the filtrate gives no trace of Ca and maintains the reaction of the neutral NH_4Cl . From 4 to 6 liters of solution are necessary (depending on the base exchange capacity) to complete the replacement. The soil is then washed with distilled water until free from chlorides, and the ammonia determined by distillation.

The NH_4 content may be checked in the following way: the same soil is saturated with the K ion, neutral *N* KCl solution being used. The total *N* is then determined on both the K- and NH_4 -saturated soils. The difference in *N* content in terms of NH_4 may be taken as the amount of *N* absorbed by the complex. The latter method, which was checked repeatedly by determining the NH_4 present in the original soil before treating with NH_4Cl , was found to be very satisfactory.

A number of soil samples of the Chenango series was treated with normal NH_4Cl solution and the ammonia absorbed, after replacing all other cations, was determined as described. Simultaneously the H ions present were determined and checked by treating the soils with *N* BaCl_2 solution and titrating the extracts and leachings with 0.02 *N* KOH to pH 6.8 to 7.0.

The results are summarized in table 1.

In general the data show that the heavier types of soil have a greater base exchange capacity than the lighter types. This, of course, is to be expected, since the heavier soils contain a large amount of the so-called colloid fraction which is chiefly responsible for the replacement reactions. This phase will be discussed fully later when the results of the experiments on the base exchange capacity of the soils from the soil fertility plots are reported. For the present an analysis of the data will be made from the standpoint of saturating the respective soils with Ca.

Let us for a moment picture in general the possible effects of adding lime to a soil depleted of its bases to various degrees through the accumulation of H ions.

The Ca ions will enter the soil complex capable of base exchange, forcing out the H and any other cations present. The Ca ions from the other calcium compounds present in the soil together with the ions going in and out from the complex possess various properties.

First, Ca is an essential element; secondly, the Ca ion is an antagonist performing an important function in balancing the physiological equilibrium of salt intake by plants, as has been shown by the researches of Osterhout (9), Loeb (8), Lipman (6), and many others; thirdly, Ca affects the degree of dispersion of the soil colloids. The divalent character of the Ca ions is of great moment in determining the degree of dispersion of the soil particles, which affect in a large measure the soil structure, increasing porosity and producing a granular structure. The sum total of the effect of the Ca ion on the col-

TABLE 1
Degree of unsaturation of soils, Chenango series

SOIL NUMBER	TYPE OF SOIL DESIGNATED BY SOIL SURVEY	REACTION OF H ₂ O EXTRACT	NH ₄ ABSORBED PER 100 GM. AIR-DRY SOIL	REPLACEABLE H IONS IN 100 GM. SOIL IN TERMS OF NH ₄	UNSATURA- TION
		pH	mgm.	mgm.	per cent
1	Loam	5.0	64.69	36.44	56.30
2	Loam subsoil	5.0	97.49	54.49	55.89
3	Silt loam	5.0	82.91	42.00	50.70
4	Silt loam subsoil	5.0	67.42	31.28	46.30
5	Sandy loam	5.0	51.02	32.18	63.08
6	Sandy loam subsoil	5.6	55.58	6.91	12.43
7	Fine sandy loam	5.8	64.69	14.00	21.64
8	Fine sandy loam subsoil	6.0	31.89	7.65	23.98
9	Fine sand	5.2	37.36	13.45	36.02
10	Fine sandy subsoil	5.2	51.93	14.72	28.35
11	Sand	4.6	35.53	20.74	58.30
12	Sandy subsoil	5.0	26.42	4.72	17.89

loidal reactions of the soil may be expressed by the suction force of the soil, as has been shown (5). It is also easily demonstrable by a determination of the heat of wetting method of Bouyoucos (2) and Anderson (1) as was shown by Parker.²

A consideration of the effect of Ca on the acid reaction of the soil solution—the elimination of the obnoxious Fe and Al ions on one hand and the locking up of phosphates and Fe on the other—is important. A good deal of work on this phase of the problem has been carried out by the authors and will be reported in a forthcoming paper of this series.

Last comes the effect of the Ca on certain biological activities in the soil,

² Paper read before the symposium on base exchange at the Chicago meeting of the American Society of Agronomy, November, 1925.

which determine the organic matter content and differentiate the relative quantities of the various fractions of organic matter, as was shown by Tyulin (10).

Table 1 shows that the total capacity of the soils for base exchange cannot be taken as a criterion for the saturation of the soil with Ca. If such a procedure should be followed it would mean that the soil would contain no cations for replacement except Ca. The deleterious effects of such a condition would be comparable to overliming, as any phosphorus fertilizers would immediately become precipitated; potassium fertilizers would be more easily leached out because the energy of replacement and adsorption of the Ca ions is greater than that of K ions.

The only logical method of applying lime is to introduce enough, in milligrams equivalent, of the H ions found by replacement and titration, to saturate the complex. Table 1 shows that each soil has a different value of base exchange capacity, with a different amount of H ions present. Each soil differs from its subsurface soil in acidity, and in general the subsurface soil is less acid, except in soils 1 and 2. The high colloid content of these soils, especially of the subsurface soils, tends to obliterate the differences.

Of course the colloid fraction is smaller in the sandy soil, and its subsurface soil has had no chance to receive basic cations from the topsoil; therefore, the podsolization in the soil profile has reached a lower horizon than in the clay soils.

In the loam soil the H ions are larger in absolute figures in the subsurface soil than in the topsoil; but relatively they are not, since the capacity for base exchange in the subsurface soil is practically one-third higher, as may be inferred from the figures on NH_4 absorbed.

The total replaceable hydrogen in the soils with a high colloid fraction increases with the degree of unsaturation. It may readily be seen that soil 5 with a pH of 5.0 will require fewer Ca ions to satisfy its unsaturated condition than soil 3; the saturation capacity for lime depends in a great measure on the base exchange capacity and on the degree of unsaturation. The only rational method of liming is, therefore, to introduce enough Ca ions to take care of the H ions present in replaceable form, and also of the hydrolytic acidity due to some of the Al and Fe compounds found in some soils.

At this point it is worth while to emphasize again the fallacy of pH readings as a criterion for measuring soil acidity. There is no correlation between the pH and the total replaceable H ions in the soil complex.

The degree of saturation or unsaturation of the top and subsurface soil gives a picture of the process of podsolization and the fate of the cations replaced.

In general the process goes on as follows: the ratio of the cations of the topsoil, where the bulk of the chemical and biological reactions takes place, is constantly changing depending on the factors of climate, tillage, and cropping. Certain cations enter the soil solution and are taken up by the plant,

others leave the soil solution to enter the complex capable of base exchange. On account of their high coefficient of replacement, the H ions from the organic acids in the process of organic matter decomposition, and inorganic acids produced by chemical interaction of the inorganic constituents with the organic acids, force out the basic cations; this action depletes the topsoil bases.

As mentioned, some of them are taken up by the plant, some are moving downward with the water and are caught by the subsurface soil, some enter the underground waters and are carried into the streams. It is easy to see that the

TABLE 2

Comparison of the lime requirement according to the Veitch method and H ion replacement with N BaCl_2 , plot soils

LABORATORY NUMBER	PLOT NUMBER*	REACTION OF WATER EXTRACT	REACTION OF N BaCl_2 SOLUTION EXTRACT	LIME REQUIREMENT (CaO) PER ACRE		
				Veitch method	H ion replacement method with†	
					N KCl solution	N BaCl_2 solution
	1914	pH	pH	pounds	pounds	pounds
1	7A	5.6	4.8		1073	1156
2	10A	5.4	4.8		1042	1010
3	11A	5.4	4.4		1844	1802
4	12A	5.6	4.8		1020	1021
	1918					
5	7A	5.4	4.8	1000	1396	1349
6	10A	5.6	5.0	1000	1082	1042
7	11A	5.0	4.4	2000	1926	1802
8	12A	5.6	5.1	1000	844	844
	1922					
9	7A	5.2	4.6	1000	1365	1365
10	10A	5.4	5.0	1000	1140	1072
11	11A	5.0	4.4	2400	2312	2200
12	12A	5.6	5.0	1000	936	880

* 7A is the control—no fertilizers; 10A received $\text{Ca}(\text{NO}_3)_2$ equivalent in terms of N to 320 pounds of NaNO_3 per acre; 11A received $(\text{NH}_4)_2\text{SO}_4$, equivalent to 320 pounds of NaNO_3 ; 12A received CaCN_2 , equivalent to 320 pounds of NaNO_3 ; all plots, except 7A, received yearly besides the nitrogenous fertilizers also 400 pounds of 16 per cent acid phosphate and 200 pounds of muriate.

† This includes the lime necessary to neutralize the acidity due to hydrolysis of Al compounds present.

monovalent cations are more quickly lost, because they do not possess so high a coefficient of replacement and absorption as the bivalent cations.

It will be recalled that the descending order of the energy of replacement and absorption of cations is: Ca, Mg, K, NH_4 , and Na, and for this reason Na goes out first.

Theoretically, therefore, the unsaturated surface soils could be greatly improved by subsoiling wherever the subsurface soil retained the leached bases. The unsaturated soils would then become the portion that would retain the

leached out bases which would replace the H ion of the underplowed unsaturated portion.

The part of the underplowed unsaturated topsoil (now subsurface soil) receiving the bulk of the replaced cations from the surface soil, which was previously a subsurface soil saturated with bases, will retain some of the cations, especially the bivalent cations, and become saturated. Thus, subsoiling an unsaturated soil would afford a means of maintaining its fertility, since the valuable basic cations would be retained in the plowed horizon and would circulate there.

Samples 5 and 6 are very striking illustrations. The topsoil has become unsaturated, whereas the subsurface soil, which has a capacity for base exchange close to that of the topsoil, has retained almost all of its bases. Such a soil is on the verge of losing its bases also from the subsurface soil. Subsoiling would bring into the plowed horizon a complex with a high capacity of valuable cations which may be utilized by the plants. The practical aspect of this problem is now under consideration and as soon as results accumulate they will be published.

From what has been said it may be inferred that the so-called "lime requirement"⁸ is linked up with the determination of the state of unsaturation. It will be of interest, therefore, to compare a few figures of the lime requirements as determined by some of the standard methods and by the replacement method.

Table 2 gives the data on the lime requirements of soils from a few of the soil fertility plots at the New Jersey Experiment Station. The data for the lime requirements [Veitch method (11)] were taken from the files of the Soil Fertility Department.⁸

The lime requirement by the replacement method was determined as follows:

Ten-gram samples of the respective soils (sieved through a 2-mm. sieve) were treated with 250 cc. of *N* BaCl₂ or *N* KCl solution, left in contact with the solution with occasional stirring for 10 days, filtered, and the filtrate was titrated electrometrically to pH 6.8 with 0.02 *N* KOH. The soil on the filter paper was washed with the respective solutions until a portion of the filtrate gave a pH reading of 6.8. From 4 to 6 liters (the soils with low colloid fractions required less than 6 liters) of the solutions were used for saturation, in other words, complete replacement. The combined filtrates were titrated and the amount of 0.02 *N* KOH used was added to the amount of the titration on the first 250 cc. of the solutions. The "lime requirement", in terms of CaO, was calculated from the total amount of alkali.

The discrepancies in "lime requirement" between the two methods as shown in table 2 are large enough to raise the question of the reliability of the Veitch method, which depends on the contact of the soil with limewater. In a way the Veitch method approaches the replacement method, but the strong reagent used introduces side reactions; and besides, the replacement process

⁸ The authors take this opportunity to express their indebtedness to Prof. A. W. Blair, who supplied the lime requirement data and placed at their disposal some of the soil samples.

could not be fully accomplished in the short interval of contact of the respective cation (Ca) with the soil. Because of this it is difficult with the Veitch method to obtain good checks on duplicate samples.

The Hopkins (4) method, which approaches still closer the replacement method for determining the "lime requirement," is open partially to the same criticisms as the Veitch method. According to the Hopkins method, the soil is treated with a neutral salt and the filtrate titrated; the titer is multiplied by a certain factor (in one place the factor is 3, in another 4, and again 2.5) to give the lime requirement.

The other methods may also be criticised from the same standpoint.

TABLE 3
Replaceable hydrogen at various time periods of extraction

PLOT NUMBER	PLOT SOILS			LABORATORY NUMBER	CHENANGO SOILS				
	Replacement hydrogen with <i>N</i> BaCl ₂ extraction after				Replaceable hydrogen with				
					<i>N</i> BaCl ₂ extraction after			<i>N</i> KCl extraction after	
	1 hour	1 day	10 days		1 hour	1 day	10 days	1 day	9 days
1914	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
7A	23.0	30.2	43.2	1	35.82	45.77	50.24	25.8	30.3
10A	25.1	31.9	33.1	2	56.66	62.66	65.33	64.0	71.7
11A	30.5	43.4	46.5	3	29.43	40.25	46.75	42.4	49.6
12A	20.0	26.6	34.6	4	33.13	36.04	38.37	57.0	63.3
1918									
7A	27.7	36.7	42.6	5	27.11	36.72	39.54	22.6	26.0
10A	20.8	27.0	36.2	6	31.57	39.47	39.47	40.0	60.0
11A	36.2	44.4	51.7	7	24.67	32.36	32.46	13.0	14.6
12A	16.0	25.0	32.3	8	23.8	33.33	33.33	33.0	33.0
1922									
7A	33.7	44.0	53.5	9	29.72	36.48	45.94	21.6	27.4
10A	26.2	30.0	37.3	10	23.45	24.69	29.62	20.8	30.8
11A	34.7	43.3	54.2	11	33.91	43.47	52.17	55.4	65.1
12A	18.4	30.5	39.6	12	46.15	73.07	73.07		

Table 3 shows how the percentage of replaceable hydrogen varies with each soil. The time of contact influences the release of the hydrogen ions, and the state of unsaturation is also an important factor.

The higher the state of unsaturation the faster the H ions are released. This is to be expected since the complex contains fewer of the other cations and the reaction is proceeding between the replaceable cations and the adsorbed H ions. For these reasons it is impossible to assign any definite factor which, when multiplied by the amount of alkali necessary to neutralize the acidity of a neutral salt solution extract in contact with the soil for any length of time, would give us the true lime requirement.

That the cations used in replacing the H ions have an influence on the speed

of replacement may be inferred from theoretical considerations; the bivalent cations with their higher coefficient of absorption and replacement ought to be more efficient in the replacement process. This has been found in general to hold true. There are, however, exceptions. The data on the relative speed of replacement by the Ba and K ions in table 3 on the Chenango soils show that the K ions replaced more H ions than the Ba ions in some samples. No explanation for such behavior can be offered. Surface relationships as influenced by the valency of the cations may enter as a factor.

At this point it will be of interest to look into the question raised by Gedroiz (3), whether the theoretical amount of lime requirement as calculated from the unsaturation of the soil coincides with the practical needs. The work of the New Jersey Agricultural Experiment Station on the limed (B) and unlimed (A) plots may offer a satisfactory explanation. The only difference in the treatment of the two series of plots is that the B plots receive an application of 2 tons of CaCO_3 every 5 years. The data on the yields from these

TABLE 4
Yield of dry matter—5-year period, 1918-1922
Calculated to acre basis

PLOT NUMBER	CORN—1918			OATS—1919		BARLEY—1920		TIMO- THY HAY 1921	TIMO- THY HAY 1921	TOTAL FOR 5 YEARS	INCREASE OVER CHECK
	Grain	Stalks	Cobs	Grain	Straw	Grain	Straw				
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
7B	2,329	1,746	404	680	1,280	568	752	650	960	8,689	Loss
8B	2,364	1,894	444	720	2,120	1,068	1,492	2,240	3,720	15,342	3,630
9B	2,316	2,406	397	440	2,440	1,244	1,796	2,960	5,040	18,599	6,887
10B	2,794	2,620	496	560	2,760	1,284	2,016	3,700	5,360	21,030	9,318
11B	2,714	2,406	478	720	2,680	1,360	2,120	3,620	4,680	20,058	8,346
12B	2,324	2,458	417	720	2,680	1,432	2,198	3,140	3,520	18,079	6,367

plots seem to indicate that such an application meets the requirements of the soil, even the one which receives as a fertilizer the acid-forming $(\text{NH}_4)_2\text{SO}_4$.

Table 4, compiled from the data on the crop yields of the plots is reported by Lipman, Blair and Prince (7), shows that 11B ranks in yield as high as any of the plots. For comparison the yields on several other plots are given in table 4. It is very possible that the low yield on the other plots is due to overliming, which probably depleted the complex from other bases and undoubtedly affected adversely the organic matter in the soil.

It may not be amiss at this point to recall that 11A, the unlimed plot corresponding to 11B, showed in 1922, just before the regular lime application was made, a lime requirement of about 2300 pounds of CaO .

A preliminary survey of the replaceable H ions in 11B shows that the complex contains replaceable H ions, even though the soil still contains carbonates of lime. This is in line with the ideas expressed by Gedroiz (3), but his fear of underliming is not well founded, as may be inferred from the

practical experiences at the New Jersey Station. It is doubtful whether a complete saturation with Ca ions would be desirable, for reasons pointed out on p. 130. The lime in the soil does not leach out to any extent and is present as a potential source, to release Ca ions into the soil solution to react with the solid phase of the complex.

SUMMARY

1. The relation of the various cations present in the soil complex (inorganic and organic) capable of base exchange is discussed.
2. The methods used in determining the base exchange capacity and the degree of unsaturation of soils are given.
3. The functions of the Ca ions in the soil-plant system are discussed from the standpoint of base exchange.
4. The relation between the state of unsaturation and the lime requirement is analyzed, and the experimental results are compared with methods for determining the lime requirement.
5. The degree of saturation and unsaturation in the layers of the soil profile indicates the possibility of utilizing subsoiling practice for the purpose of preserving the bases that are being leached out from the surface soils.
6. It is shown that an application of lime as indicated by the replacement method of lime requirement, is sufficient for the best results.

REFERENCES

- (1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.* 28: 927-935.
- (2) BOUYOUCOS, G. J. 1925 The heat of wetting as a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153-162.
- (3) GEDROIZ, K. K. 1924 Soils unsaturated with bases, etc. *Zhur. Opytn. Agron.* (Russian) 22: 3-28.
- (4) HOPKINS, C. G., KNOX, W. H., AND PETTIT, J. H. 1913 A quantitative method for determining the acidity of soils. *U. S. Dept. Agr. Bur. Chem. Bul.* 73: 114-118; see also Hopkins: *Soil Fertility and Permanent Agriculture*, p. 627, 1910.
- (5) JOFFE, J. S., AND MCLEAN, H. C. 1926 Colloidal behavior of soils and soil fertility: II. The soil complex capable of base exchange and soil acidity. *Soil Sci.* 21: 181-195.
- (6) LIPMAN, C. B. 1913 The theory of antagonism of salts and its significance in soil studies. *Proc. Soc. Prom. Agr. Sci.* 1913: 1-18. (See also C. B. Lipman's other publications from Univ. Calif.)
- (7) LIPMAN, J. G., BLAIR, A. W., AND PRINCE, A. L. 1925 Field experiments on the availability of nitrogenous fertilizers 1918-1922. *Soil Sci.* 19: 57-79.
- (8) LOEB, J. 1900 Antagonistic action of salts and balanced solutions. *Amer. Jour. Physiol.* 3: 434. (See also Loeb's publications in *Jour. Gen. Physiol.* since 1918.)
- (9) OSTERHOUT, W. J. V. 1919 Antagonism between alkaloids and salts in relation to permeability. *Jour. Gen. Physiol.* 1: 515-519. (See also Osterhout's numerous papers in *Bot. Gaz.* since 1906; in *Jour. Biol. Chem.*, *Science*, and other publications.)
- (10) TYULIN, A. F. 1923 Influence of lime on the decomposition of organic matter in soils. *Trans. Inst. Fert.* (Russian) 14: 3-12.
- (11) VEITCH, F. P. 1902 The estimation of soil acidity and the lime requirement of soils. *Jour. Amer. Chem. Soc.* 24: 1120-1128.

A STUDY OF THE FACTORS INFLUENCING THE EFFICIENCY OF DIFFERENT FORMS OF NITROGEN AS RELATED TO SOIL TYPE AND CROPPING SYSTEM IN THE ATLANTIC COASTAL PLAIN REGION: PART I¹

ARTHUR MATTHIAS SMITH²

University of Maryland

Received for publication April 28, 1926

INTRODUCTION

The economic utilization of nitrogen fertilizers is one of the outstanding problems of the market gardeners and truck farmers on the sandy soils of the Atlantic Coastal Plain. The Norfolk, Sassafras, Ruston, Keyport, and Elkton soils in particular contain relatively small amounts of nitrogen, and are usually coarse in texture. Since in many areas the subsoil contains even more sand than the surface soil, these soils are frequently subject to excessive leaching from both winter and summer rains. Especially in the tide-water regions of Maryland and Virginia, where the permanent water-table is close to the surface soil, large amounts of nitrogen are lost annually by leaching.

That the supply of available nitrogen is one of the important problems to farmers in the Atlantic Coastal Plain may be realized from the fact that an application of one ton per acre of a 7-6-5 or a 5-8-5 (NH_3 - P_2O_5 - K_2O) commercial fertilizer is the standard practice for early potatoes in many sections. These fertilizers cost on the average from \$45.00 to \$52.00 per ton. This has been estimated by economists of the United States Department of Agriculture (76) to be from 26 to 33 per cent of the total cost of producing this crop. For many other truck crops applications of from 1000 to 1500 pounds of similar fertilizers per acre are made.

Since the severe summer showers and heavy winter rains in this region tend toward the excessive leaching of nitrate nitrogen; the farmers have demanded large proportions of organic nitrogen materials in the mixed fertilizers, with a consequent increase in the basic cost of the same.

In this investigation an attempt is made to study some of the fundamental factors involved in the use of complete commercial fertilizers on these soils, with special reference to the proportion of the nitrogen derived from inorganic and organic nitrogen fertilizer materials.

¹ Summarized Introduction and Historical Review, of a Thesis submitted to the Graduate Faculty of the University of Maryland, June, 1925, in partial fulfillment of the requirements of the degree of Doctor of Philosophy. Part II is a detailed report on closely related field-plot experiments, and will be published at a later date with subsequent data.

² The writer takes this opportunity to thank Dr. A. G. McCall, under whose direction this investigation was conducted, for his helpful suggestions, kindly criticisms, for the facilities made available, and for reading the manuscript. Thanks are also extended to Director H. J. Patterson for his kindly cooperation.

HISTORICAL

FACTORS INFLUENCING THE RATE OF NITRIFICATION

The effect of temperature on nitrification

The work of numerous investigators has shown that nitrification will take place in soils at all temperatures between 15° and 40°C. In particular, that of Jacobs, Allison and Braham (45), and of Panganiban (63) indicates that the optimum temperature is approximately 35°C.

The consensus of opinion from the work of Lemmerman and Wichers (50); Fraps (28); Whiting and Schoonover (80); Shonbrunn (70), Blair and Prince (10); Gowda (35); and others, is that seasonal variations in the rate of nitrification are due largely to temperature, moisture, and other seasonal changes in the soil.

Moisture relations

Harris and Butt (40) found that a soil-moisture content of 23 to 28 per cent was optimum for nitrification. The work of Coleman (24), and of Jacobs, Allison and Braham (45) indicates that for most soils 50 to 60 per cent of the water-holding capacity is the optimum. Sachs and Austin (65), Albrecht (1), The Nebraska Agricultural Experiment Station (62), Munter (59), Carpenter and Bose (19), and Hall (38), all found that tillage practices designed to conserve moisture in field soils during the summer, increased the amount of nitrification.

Loss by leaching

The work of Garretson (32) and of Geilman (33) shows very little leaching of the ammonia form of nitrogen; most of the nitrogen lost in this manner being nitrate. Blair (10) found no more leaching when nitrate of soda was added to the soil used than when organic ammoniates were added. This brings up the question of soil texture and chemical composition in relation to the influence of sodium. Harrison (41) showed that a fairly heavy soil to a depth of 3 to 4 feet is necessary to prevent excessive leaching. The work of Wilson (81), and of Fraps (28) illustrates the value of crops and manures in conserving soil nitrates.

The previous work indicates that the leaching of soil nitrates is the resultant between the factors of soil texture, soil composition, climate, and the cropping and manuring systems.

The effect of soil reaction

The wide range of H-ion concentrations in soils in which nitrification is reported to have proceeded vigorously, leads one to believe that other soil factors such as moisture, temperature, buffer substances, i.e. organic matter, colloidal clay and zeolites, and microorganisms as well, may, in many instances, outweigh the influence of either an unfavorable OH- or H-ion concentration. This view is supported by the work of Fred and Graul (30), Garder and Hagum (31), Meek and Lipman (57), Barthel and Bengston (8), Fraps (28), Stevenson (73), Chardon-Placios (20), Blair and Prince (11), Shedd (69), Waksman (79), Munter (60), Murphy (61), Albrecht (3), and Denison (25).

For mineral soils the optimum reaction appears to be at about pH 7.0; although maximum values of OH-ion concentration of 13.0 and minimum values of pH 5.4 have been recorded. The work of Fraps (28), Murphy (61), and many others indicates that liming the soil favors nitrification; an exception being the work of Blair and Prince when using sulfate of ammonia as a fertilizer. Minimum values of pH 4.0 have been observed in peat soils. This is in line with Waksman's researches and explanation of the influence of various buffering materials.

The influence of salts on nitrification

The relation of salts to the nitrification process is of importance with respect to the influence of the non-nitrogenous fertilizer salts on the rate of availability of the nitrogen con-

tained in the fertilizer and also in the soil. Greaves (36) found that in alkali soils many salts increased the rate of nitrification. Erdman (27) found that normal applications of gypsum had no effect. Brown and Gowda (18) found that phosphates and nitrate of soda increased nitrification. The work of Fraps (28) on Texas soils showed phosphates to be more effective than potash in stimulating nitrifying bacteria. R. S. Smith (71) found that potassium sulfate stimulated and potassium chloride decreased the rate of nitrification.

Influence of the source of the nitrogen on nitrification

In the work of Jacobs, Allison and Braham (45) light applications of cyanamide were entirely nitrified in 5 days; and applications of 216 pounds per acre were completely transformed to nitrate in 10 days. They also found that small quantities of dicyano-diamide did not delay the nitrification of urea, but did delay the transformation of ammonium sulfate for 210 days. Littauer (54) found that urea was nitrified best in a loam soil; and, although drouth retarded the rate, increases in moisture above the average water capacity produced no essential changes in the rate of change to nitrate.

It is evident from the literature that the presence or absence of certain impurities in urea, as determined by the process of manufacture, largely determines the rate of nitrification.

Brenchley and Richards (16) found that all the nitrogen from finely divided dried blood was nitrified after 15 weeks. In the same time 26 per cent of that in slate-bed sewage sludge and 66 per cent of that in activated sludge has been changed to nitrate.

PLANTS AS INDICATORS OF THE AVAILABILITY OF NITROGEN FROM VARIOUS MATERIALS

So much experimental work has been published on various crops as indicators of nitrogen availability, that it is possible to cite only a few investigations bearing directly on some phases of the present problem, for example: the work of Schneidewind, Meyer and Munter (68); Hasselhof, Liehr and Fluhrer (42); Kuyper (48); Chevalier (21); Allison, Braham and McMurtey (4); Prescott (64); Allison, Vliet, Skinner, and Reid (5); Brenchley and Richards (16); Brown (17); Lemmerman and Eckl (49); Truffaut and Bezssonoff (74); Woods (83); Lipman and Blair (52); and Martin (56).

The consensus of opinion seems to be that crop response to different nitrogenous materials depends so largely on soil texture and composition, climatic factors, length of growing season and the root system of the crop, that no one material or type, of material is best adapted to all, or even to a large proportion of soils and crops. It has been largely a problem of determining the effects of these various influences on each nitrogen fertilizer, and thus learning its adaptability for any particular soil and crop combination.

The general conclusion seems to be that the water-soluble forms of nitrogen are best adapted to heavy soils and to short or cold growing seasons. For sandy soils, long growing seasons, and high soil temperatures, the soluble and insoluble organic nitrogen materials and the ammonia salts seem better adapted than the nitrate forms.

NITRIFICATION AS A MEASURE OF THE AVAILABILITY OF NITROGEN FOR PLANT GROWTH

According to the work of Bonazzi (13), and from a consideration of the processes for the synthetic production of ammonia and nitrates, the oxidation of ammonia in soils is accomplished by the catalytic activity of iron as part of a biological intracellular mechanism, through which the inert oxygen of the air is activated. Miyake and Soma (58) have shown that the process of nitrification as a whole resembles an autocatalytic mono-molecular bio-

chemical reaction; and that the increase of nitrates in soils is in accord with the formula for this type of reaction.

Hasselhof (42); Robinson, Winter, and Miller (65); and others, have shown that although the neutral and alkaline permanganate methods shed some light on the availability of insoluble, but easily hydrolyzable, organic nitrogenous compounds, they do not yield true values when compared with plant-culture experiments. The time consumed and the tedious processes involved make bacteriological and nutritional methods impractical for routine use in control laboratories. However, these latter methods are of real worth in establishing true values for the more rapid chemical methods in present use, or those to be devised in the future.

The work of Lipman and Burgess (51), Bizzell (9), Fraps (29), Waksman (78), and others, seems to show that nitrification tests under standard conditions are the best biological method for determining the availability of nitrogen in fertilizer materials; unless crop tests can be made under the soil and climatic conditions existing in the field where these materials are to be used. The results obtained by Lipman and Burgess (51) and by Waksman (78) in comparing ammonification and nitrification tests with crop growth experiments, seem to indicate that ammonification tests fail to yield real or consistent values over a wide range of conditions, such as exist in various field soils.

Although the writer is fully conversant with the work recently brought into prominence concerning the availability of the ammonia form of nitrogen to economic crop plants, he is inclined to agree with the findings of these investigators cited above. Although certain plants whose ecological habitat is found in wet, poorly drained, or very acid soils may be shown to take up appreciable quantities of nitrogen in the form of ammonia; the bulk of the evidence from practical farm experience, field plot experiments, pot culture studies, and bacteriological research shows that of the simple transformation products of organic nitrogen, nitrate is the only stable form in the soil; and that the proportion of nitrate to ammonia nitrogen found in most well drained soils proves conclusively that plants do absorb greater quantities of the nitrate form than of the ammonia form of nitrogen. For these reasons nitrification tests were adopted as the criterion of nitrogen availability in the present investigation.

EXPERIMENTAL

A COMPARISON OF VARIOUS NITROGENOUS FERTILIZER MATERIALS WITH RESPECT TO THE RATE OF NITRIFICATION AND THE LEACHING OF NITRATES FROM NORFOLK SANDY LOAM SOIL

The surface soil used in these experiments was secured from the experimental field at Snow Hill, Maryland. The data from preliminary mechanical and chemical analyses are given in table 1.

Sulfate of ammonia, urea, dried ground fish, high-grade packing-house tankage, and activated sewage sludge were compared with respect to their rate of nitrification in this soil. The urea was of the usual commercial grade now being sold in this country by the Badische Anilin und Soda Fabrik. The sulfate of ammonia, fish, and tankage were the usual commercial materials. The activated sewage sludge was obtained from Milwaukee, Wisconsin, the material having been produced by the method described by Kadish (46). Commercial nitrate of soda was used in the leaching experiments, and as a control on the nitrate absorption of the soil in the rate of nitrification studies.

In order to obtain an equitable basis for comparison, the organic materials were ground to approximately equal fineness, and to a degree possible under commercial conditions. Sieve tests were then made and the total nitrogen was determined, the results are shown in table 2.

Series I. The influence of soil moisture on the rate of nitrification

Each of the nitrogenous materials listed in table 2 was used in turn as the sole source of nitrogen in a complete fertilizer containing 7 per cent ammonia, 6 per cent phosphoric acid, and 5 per cent potash. In all cases the phosphorus and potassium were derived from 16 per cent acid phosphate and 50 per cent sulfate of potash, respectively. Of this fertilizer, 4-gm. portions containing

TABLE 1
Mechanical analysis determinations, Norfolk Sandy Loam

Mechanical analysis:*	
Fine gravel and coarse sand.....	per cent 16.0
Medium sand	per cent 31.8
Fine sand.....	per cent 36.4
Very fine sand.....	per cent 4.2
Silt.....	per cent 9.0
Clay.....	per cent 2.1
<hr/>	
Water-holding capacity	per cent 27.1
Total nitrogen	per cent 0.041
<hr/>	
H-ion concentration	pH 6.4
Lime requirement (Veitch method) 1500 pounds CaCO ₃ per acre	

* Method of Bureau of Soils, U. S. Dept. Agr. (15).

TABLE 2
Analyses of nitrogenous materials used

MATERIALS	NITROGEN	MECHANICAL ANALYSIS					
		Coarser than 20 mesh	20-40 mesh	40-60 mesh	60-80 mesh	80-100 mesh	Finer than 100 mesh
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Dried ground fish.....	9.6	0.2	4.8	29.8	19.0	8.2	37.4
Packing house tankage.....	10.3	0.1	1.4	24.0	17.7	10.2	46.6
Sewage.....	5.1	.	2.0	37.2	16.9	8.4	35.5
Urea.....	45.3
Sulfate of ammonia.....	25.2
Nitrate of soda	14.9

230.4 mgm. of nitrogen each, were added to 4 kgm. of sifted air-dry soil contained in 1-gallon glazed pots. These additions correspond to a field application of 2000 pounds of fertilizer per acre.

The rate of nitrification of sulfate of ammonia, urea, fish, and tankage was compared at soil-moisture contents of 10, 20, 30, 40, 50, 60, and 70 per cent of the total water-holding capacity of the soil as determined by the Hilgard method (43-a). At the lowest moisture content, 108 gm. of water was added

TABLE 3
The effect of soil-moisture on the rate of nitrification

MOISTURE CONTENT	POT NUMBER	SOURCE OF NITROGEN	NITRATE-NITROGEN IN AIR-DRY SOIL							
			December 17	December 31	January 14	January 28	February 11	February 25	March 11	March 25
			0 days	14 days	28 days	42 days	56 days	70 days	84 days	98 days
10 per cent of water-holding capacity	1	Sulfate of ammonia	p.p.m. 1.9	p.p.m. 5.5	p.p.m. 6.9	p.p.m. 6.8	p.p.m. 6.7	p.p.m. 7.5	p.p.m. 5.7	p.p.m. 8.2
	2	Urea	0.6	5.6	7.1	6.5	7.1	7.5	8.2	11.1
	3	Dried ground fish	1.0	6.0	6.9	7.2	7.4	9.2	6.5	9.8
	4	Tankage	2.4	5.8	7.1	6.1	5.7	7.0	5.1	7.1
20 per cent of water-holding capacity	5	Sulfate of ammonia	1.9	2.6	7.2	12.4	21.4	30.1	22.7	41.7
	6	Urea	0.6	4.7	13.7	15.2	22.4	29.3	29.3	44.9
	7	Dried ground fish	1.0	5.4	11.1	14.7	20.4	19.6	26.8	27.1
	8	Tankage	2.4	5.1	11.1	14.9	27.6	21.2	28.3	31.3
30 per cent of water-holding capacity	9	Sulfate of ammonia	1.9	3.4	12.7	14.2	22.2	34.4	34.4	57.7
	10	Urea	0.6	4.3	16.4	27.8	32.2	37.9	43.4	52.1
	11	Dried ground fish	1.0	2.0	14.8	21.7	28.1	53.6	37.5	43.6
	12	Tankage	2.4	4.6	16.2	19.9	23.7	39.9	29.4	45.7
40 per cent of water-holding capacity	13	Sulfate of ammonia	1.9	2.9	14.8	25.9	36.8	41.7	40.1	37.5
	14	Urea	0.6	8.8	34.5	32.0	36.8	54.0	36.2	30.0
	15	Dried ground fish	1.0	4.5	13.2	24.4	37.5	53.6	34.3	47.8
	16	Tankage	2.4	5.6	18.5	27.4	38.3	50.0	31.3	49.3

	17	Sulfate of ammonia	1.9	6.1	17.8	30.5	49.3	58.6	45.7	73.5								
50 per cent of water-holding capacity	18	Urea	0.6	6.5	19.8	35.4	59.5	67.0	21.3	51.7								
	19	Dried ground fish	1.0	4.0	15.8	25.5	47.9	47.5	35.4	37.5								
	20	Tankage	2.4	6.8	18.7	28.2	46.6	52.8	28.9	22.7								
	21	Nitrate soda	65.2	20.3	73.5	69.5	80.7	85.2	35.7	60.0								
	22	Sewage	1.7	5.2	17.6	25.0	28.6	33.3	21.3	30.6								
	23	0-6-5	1.9	6.3	13.7	15.8	14.7	19.9	12.3	16.0								
60 per cent of water-holding capacity	24	Sulfate of ammonia	1.9	5.8	18.7	37.9	48.7	65.8	53.6	51.7								
	25	Urea	0.6	7.4	31.1	66.4	62.5	76.5	60.0	62.5								
	26	Dried ground fish	1.0	7.7	17.5	41.2	41.4	56.8	29.3	44.1								
	27	Tankage	2.4	7.1	20.9	40.1	39.5	50.3	21.1	40.5								
70 per cent of water-holding capacity	28	Sulfate of ammonia	1.9	7.8	20.0	42.9	37.5	54.0	45.2	32.1								
	29	Urea	0.6	6.8	29.9	67.0	49.3	67.0	69.5	69.5								
	30	Dried ground fish	1.0	5.1	17.3	42.9	41.4	44.1	48.4	27.3								
	31	Tankage	2.4	7.3	20.6	40.3	37.1	41.9	43.4	28.2								
Mean air temperature			{ °C. °F.		14.0	57.1	15.1	59.7	16.1	61.8	18.0	64.1	17.1	63.3	18.1	64.8	18.0	63.9

to 4000 gm. of soil, while at the highest, 761 gm. of water was added. The experiment was conducted in the greenhouse for a period of 98 days from December 17 to March 25, during which time the pots were neither covered nor shaded. The pots were weighed twice each week, and distilled water was added to replace the loss by evaporation. After each sampling the soils were cultivated to a depth of $\frac{1}{2}$ inch.

At 50 per cent of the water-holding capacity three additional fertilizer treatments were employed. In one of these, activated sludge was used as the nitrogenous material. Another, in which nitrate of soda was used, served as a control pot for measuring nitrate absorption by the soil and the utilization of nitrates by soil organisms. The third, to which no nitrogen was added, served as a control to measure the rate of nitrification of the original soil nitrogen.

In order to obtain representative samples, borings from the top to the bottom of each pot were made at 14-day intervals. Seventy-gram samples of the moist soil were dried in the oven at 50°C. for 15 hours, and 50-gram portions

TABLE 4

Time-periods required for the nitrate nitrogen to equal the total nitrogen applied in the fertilizer

MATERIALS	SOIL-MOISTURE AS PER CENT WATER-HOLDING CAPACITY						
	10 per cent	20 per cent	30 per cent	40 per cent	50 per cent	60 per cent	70 per cent
	days	days	days	days	days	days	days
Sulfate of ammonia	98	..	70	70	70*
Urea	98*	70*	56	42	42
Dried ground fish	70*	70*	70	..
Packing house tankage	70*	70*	70*	..

* Amounts slightly less than 57.6 p.p.m.

of the dried soil were then extracted with 250 cc. of distilled water. Nitrates were determined throughout by the phenol-disulfonic acid method as modified by Harper (39).

The results of the nitrate determinations for each 14-day period are shown in table 3, in which the nitrate-nitrogen is expressed as parts per million of air-dry soil.

An inspection of table 3 shows that, in general, for the first 42-day period the rate of nitrification increased in direct proportion to the increase in soil moisture. For the 42 to 98-day period there was a direct increase in nitrification with increasing soil moisture up to 50 per cent of the water-holding capacity of the soil, except in the case of urea, which yielded a high nitrate content at both 60 and 70 per cent of the water-holding capacity.

At the lowest moisture content very little nitrification took place from any of the materials added. At the 20 per cent level and, in fact, at all of the higher moisture contents, urea and sulfate of ammonia nitrified more rapidly and gave higher nitrate accumulations than either fish or tankage.

Considerable variation in the nitrate accumulation for all of the materials may be noticed at the higher soil moisture contents after the 56-day period. This periodicity during short intervals has been noted by numerous investigators. Löhnis in particular (54) has observed similar fluctuations due to biological factors, such as the utilization of nitrates by microorganisms other than the nitrate bacteria. In the present experiment the fluctuations cannot be explained satisfactorily on the basis of differences in soil moisture, temperature, or H-ion concentration, and accordingly are probably due to nutritional differences caused by the various rates of decomposition of the soil organic matter as influenced by the decomposition products and the nitrification of the materials added.

The theoretically possible concentration of nitrate nitrogen as calculated from the total nitrogen contained in the soil and fertilizer, is 467.6 p.p.m., of which 57.6 p.p.m. was added in the fertilizer. It is interesting to observe for the various soil-moisture contents the time required with the different materials for the nitrate-nitrogen to equal the nitrogen added in the fertilizer. These observations are presented in table 4.

From this summary it may be noted that in this soil, under the conditions of the experiment, the optimum soil-moisture content for the nitrification of these four materials lies between 50 and 60 per cent of the water-holding capacity. It will also be noted that a greatest total nitrate accumulation occurred at somewhat higher moisture contents for sulfate of ammonia and for urea than for either fish or tankage. The rate of nitrification in this soil at moisture contents of 30 per cent of the water-holding capacity or less is exceedingly slow. Of the four materials, urea nitrified most readily, and, with the exception of nitrate of soda and of sulfate of ammonia at certain moisture contents, gave the highest accumulation of nitrates.

It is not to be supposed that all of the nitrate-nitrogen was derived from the fertilizer materials. The work of R. S. Smith (71), Gowda (35), and others has shown that both phosphates and potassium salts stimulate nitrification of the original nitrogen contained in the soil. Table 3 shows numerous instances of nitrate accumulation greater than the amount of nitrogen supplied in the fertilizer. It is interesting to note that pot 27, which received nitrate of soda, showed not only the greatest accumulation of nitrate nitrogen, but also the largest excess over that supplied in the fertilizer. This shows a direct stimulation of nitrification by this material. The fluctuation in nitrate nitrogen in this pot at the different time intervals would indicate that nitrate of soda also stimulated the growth and activity of the other soil organisms.

After the first 28 days at 50 per cent of the water-holding-capacity of the soil, the nitrification of sewage sludge took place very slowly. After the first 14 days, the pot receiving phosphate and potash only had a much lower nitrate content than any of those receiving nitrogenous fertilizers.

The H-ion concentration of the soil as determined colorimetrically at the beginning of the experiment was pH 6.4. There was no noticeable difference

TABLE 5
The effect of soil temperature on the rate of nitrification

POT NUMBER	SOURCE OF NITROGEN	NITRATE-NITROGEN IN AIR-DRY SOIL							
		December 17	December 31	January 14	January 28	February 11	February 25	March 11	March 25
		0 days	14 days	28 days	42 days	56 days	70 days	84 days	98 days
Cold room	Sulfate of ammonia	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.	p p m.
	Urea	1 9	3 4	11 2	9 7	10 4	15 8	39 7	49 0
	Dried ground fish	0 6	3 4	9 9	10 3	10 5	18 8	48 4	63 0
	Tankage	1 0	1 9	13 2	8 8	10 5	16 7	41 0	45 7
	Sewage	2 4	2 4	10 7	10 3	11 8	19 2	41 0	48 7
Shaded green house	0-6-5 check	1 7	2 5	9 5	9 7	11 0	18 3	31 9	28 9
	Sulfate of ammonia	1 9	3 5	11 2	10 8	12 4	13 6	17 1	14 4
	Urea	1 9	3 9	14 1	36 8	37 9	56 4	44 4	75 0
	Dried ground fish	0 6	5 1	25 8	50 0	50 3	62 5	62 0	73 5
	Tankage	1 0	5 5	16 9	39 9	36 4	51 4	48 1	61 5
Not shaded green house	Sewage	2 4	5 6	20 4	37 1	37 9	58 6	44 9	79 0
	0-6-5 check	1 7	7 4	20 8	30 5	31 3	32 6	35 1	34 9
	Sulfate of ammonia	1 9	2 8	14 4	18 5	14 9	24 8	18 3	22 1
	Urea	1 9	6 1	17 8	30 5	49 3	58 6	45 7	73 5
	Dried ground fish	0 6	6 5	19 8	35 4	59 5	67 0	21 3	51 7
	Tankage	1 0	4 0	15 8	25 5	47 9	47 5	35 4	37 5
	Nitrate soda	2 4	6 8	18 7	28 2	46 6	52 8	28 9	22 7
	Sewage	65 2	20 3	73 5	69 5	80 7	35 2	35 7	60 0
	0-6-5 check	1 7	5 2	17 6	25 0	28 6	33 3	21 3	30 6
		1 9	6 3	13 7	15 8	14 7	19 9	12 3	16 0

Warm rose house	38	Sulfate of ammonia	1 9	4 7	19 3	52 5	54 8	52 5	70 1	117.2
	39	Urea	0 6	5 9	29 8	75 0	70 8	75 0	81.5	61.0
	40	Dried ground fish	1 0	3 2	23 3	48 1	50.7	50 0	65.2	79.8
	41	Tankage	2 4	6 8	29 2	51 7	50 7	49.3	57.7	90.4
	42	Sewage	1 7	6 7	22 3	41 7	35 7	41.0	30.5	60.0
	43	0-6-5 check	1 9	6 3	11 7	18 6	23 8	20 8	12 5	26.1
Mean air temperature (main greenhouse)			{ °C. °F		14 0	15 1	16 1	17 1	18.1	18 0
					57 1	59 7	61 8	63 3	64 8	63 9

in the H-ion concentration after 98 days with respect to the various moisture contents. In the soil receiving the fertilizer containing sulfate of ammonia, the initial H-ion concentration was pH 6.0. After 98 days the average pH values for the soils receiving the various sources of nitrogen were: sulfate of ammonia, 5.6; urea, 5.7; fish and tankage, 5.9. As might have been predicted from the mechanical analysis, given in table I, the buffer action of this soil is relatively low. The temperature relations will be discussed in connection with the next series of experiments.

Series II. The influence of soil temperature on the rate of nitrification

The duration, periods of sampling, containers, weight and type of soil employed, and the analysis and rate of application of the fertilizer were the same in Series II as in the preceding series.

The following materials were used as the sole source of nitrogen in the 7-6-5 complete fertilizer: sulfate of ammonia, urea, dried ground fish, packing-house tankage, and activated sewage sludge. A check treatment of phosphate and potash only was included in each group. To each pot, water was added to 50 per cent of the water-holding capacity of the soil, and the loss by evaporation was replaced twice each week.

Four groups of 6 pots each, treated as indicated above, were kept for 98 days at different temperatures. The first group, pots 44 to 49 as shown in table 5, was kept in a cold room, which received daylight through several windows. The second group, pots 32 to 37, was kept on a shelf under a stone-top table in the main greenhouse and was protected at all times from the warming effect of direct sunlight. The third group, pots 17 to 23, was placed in the main greenhouse and exposed to sunlight. This group is included in Series I as the 50 per cent water-holding capacity treatment. The fourth group, pots 38 to 43, was placed in a rose greenhouse where the temperature was maintained at a higher and more uniform temperature than in the house first mentioned.

No attempt was made to control the temperature of the soils, but frequent thermometer readings showed the following temperature conditions. The soil-temperature in the cold room ranged during the first 56 days from 3° to 12°C., and for the last 42 days from 12° to 17°C. The mean temperature for each bi-weekly period in the main greenhouse as recorded by a thermograph, is given at the foot of tables 3 and 5. The weekly average of the maximum air temperatures ranged from 17.5° to 27°C., and the weekly average minimum from 7° to 13°C., giving an average diurnal change of 10.5° to 13°C. The soil temperatures, of course, did not undergo either of these extreme fluctuations, having at most times a lag of 3 to 5°C. for rising and falling temperatures. The soil in pots 32 to 37 shaded by the stone-top table was at all times during sunny days from 2° to 3°C. cooler than pots 17 to 23 placed on top of the wooden table. The soils in pots 38 to 43 in the warmer greenhouse averaged at all times from 3° to 5°C. warmer than pots 17 to 23 in the main

unshaded greenhouse. During periods of exceedingly cold weather these soils frequently had a temperature 8°C. higher than those of the main greenhouse.

The object in comparing the rates of nitrification of these materials at different temperature levels was to ascertain, if possible, their adaptation for both early and midseason crops.

The results of the nitrate determinations at successive 14-day intervals are shown in table 5.

It may be seen from the data that in the pots placed in the cold room, the rate of nitrification during the first 70 days was slow for all of the nitrogenous materials employed, being at the most only 50 per cent more than for the soil alone. There was, however, a gradual nitrate accumulation at this low temperature level. During the last 28 days, as the temperature increased with the rising outdoor temperatures, there was an appreciable nitrate accumulation in all cases, equaling with some of the materials, the amounts shown in the main greenhouse where higher temperatures prevailed. The rapidity of nitrification at this low temperature level was in the following order: urea, then tankage, sulfate of ammonia, and fish closely grouped, activated sewage sludge, and no applied nitrogen. These results would indicate that urea might have a slight advantage over the other materials as a source of nitrogen for early spring or winter crops.

A comparison of the data for the shaded and unshaded soils in the main greenhouse shows considerable variation in the effect of the shading. Although in general the slightly higher temperatures resulting from the exposure to sunlight induced a slightly more rapid nitrification at the start, and with the exception of the activated sewage sludge, yielded a higher nitrate accumulation at the 56-day period, the influence of the slight temperature difference is not consistent. Of two possible explanations one is, that the greater loss of water by evaporation from the group exposed to sunlight may have offset the advantage of the slightly higher temperature attained. In Series I it is shown that 50 per cent of the moisture holding capacity approaches the lower limit of the optimum water content for nitrification in this soil. Accordingly, the more rapid evaporation would not provide more favorable moisture relations or stimulate nitrification through increased soil aeration.

In view of the data for Series I, however, in which there was a fluctuation in the nitrate accumulation from these materials at all soil-moisture levels of 30 per cent of the water-holding capacity and higher, and under the same temperature range as for numbers 17 to 23, the explanation does not seem to lie in a difference in moisture content. It will be observed that the nitrate accumulations in the soils exposed to sunlight showed more extreme fluctuations than those in the shaded soils, although the variations in the two groups agree in general with respect to the time periods of sampling. From the data it appears that the slightly higher temperatures attained during sunny days in the group exposed to sunlight, stimulated the growth and activity of the entire microbiological flora of this soil, to the extent of utilizing a larger portion

of the accumulated nitrates, than was the case in the shaded soils. This hypothesis is strengthened by the fact that for practically every treatment in the shaded, as well as in the unshaded soils, there was an actual *decrease* in nitrate accumulation at the end of the bi-weekly periods during which there was an *increase* in the mean temperature. Thus there was very little increase in nitrate accumulation in the shaded soils from the 42- to the 56-day period during which time there was an increase in mean temperature of 1.9°C . Although the soils exposed to sunlight showed a consistent accumulation of nitrates from all the materials under comparison for the first 70 days, during the next 14-day period there was a decided decrease in nitrates in both the shaded and unshaded soils. At the same time the mean temperature increased 1.0°C . During the final 14-day period immediately following, there was again a marked increase in nitrate accumulation in both shaded and unshaded soils in nearly every case, while the mean temperature remained practically constant.

That this fluctuation in nitrate accumulation is due to the utilization of nitrates by soil microorganisms, as stimulated by a slightly increased mean temperature, is further indicated by referring in table 3 to the analyses at the 84-day period. The nitrate accumulations from urea, fish, and tankage at soil moisture levels of 30 and 40 per cent of the moisture-holding capacity equaled or exceeded the accumulations from the same materials during the same time in the soils moistened to 50 per cent, and, for some materials, to 60 per cent of the moisture-holding capacity. This would indicate that the fluctuations in the amounts of nitrate accumulations were not due to the more rapid evaporation of water at the slightly higher mean temperature levels. It may also be observed from table 3 that increasing mean temperatures did not offset the unfavorable conditions due to supra-optimum moisture relations in the group of soils moistened to 70 per cent of the water-holding capacity. Had the loss of water by evaporation been the cause of the fluctuations observed, these soils should have undergone an increase, rather than a continued decrease, in nitrate content during the final 28 days. In fact the amounts of moisture lost were practically negligible, in view of the weight of soil employed—4000 gm.—and of the semi-weekly additions of water.

In considering the data from the soils in the warm greenhouse it will be noticed that the fluctuations in accumulated nitrates are less evident and more irregular than for the soils kept in the main greenhouse. It will also be noticed that the time periods of fluctuation in nitrate accumulation do not coincide closely with those of the other soils. This might be expected, as there was very little correlation between the climatic factors existing in the two greenhouses. In the latter, used chiefly for the winter forcing of roses, a uniformly warm temperature was maintained. It may be observed from table 5 that in practically every case the rate of nitrification and nitrate accumulation was greater for the same materials at the same time intervals at these higher temperatures (by 3° to 5°C .), than in the main greenhouse at the lower tem-

peratures. This clearly shows the favorable influence of increased temperatures on nitrification.

It should be observed that in no case did the temperatures attained in these studies reach the optimum temperatures of 30° to 35°C. for nitrification as reported by Jacob, Allison and Braham (45), and by Panganiban (63). The purpose of this experiment was to compare at rather low soil temperatures the rate of nitrification of the nitrogen supplied by these materials, in order to simulate, as far as possible, the temperature conditions existing in field soils of this type at the time of planting early truck crops. The soil-temperatures of the experimental field at Snow Hill, Maryland, were recorded by thermographs during the growing season of 1924, for which the data on crop yields are included in part II of this paper.

For consecutive 14-day periods up to a total of 70 days from the date of planting early potatoes, the bi-weekly mean temperatures of the field soil at a depth of 6 inches were 5°, 6°, 3.5°, 2°, and 1°C. less than those recorded for corresponding periods in the main greenhouse. The other factors encountered in field conditions will be discussed in connection with the field-plot data.

Although the nitrogen from tankage nitrified slightly *more rapidly* than from fish at the higher temperatures, the nitrate accumulation from these two materials does not seem consistently different. Urea nitrified *more rapidly* than any of the other materials compared, except at the lowest temperature level, for which, however, it gave the highest nitrate accumulation after 84 days.

Sulfate of ammonia nitrified less rapidly than urea, fish, or tankage during the first 4 to 6 weeks. At the higher temperatures, however, it nitrified more rapidly than did the activated sewage sludge. Attention is called in table 3 to the high nitrate accumulation from sulfate of ammonia at the end of the 98-day period. There was a direct correlation between the nitrate accumulation from this material and the H-ion concentration, the lowest pH value, 5.2, accompanying the maximum nitrate content from the sulfate of ammonia treatment. An interesting problem is suggested regarding the permanence of the nitrate accumulations from sulfate of ammonia with respect to the effect of low pH values on the utilization of nitrates by soil microorganisms.

In general after the first 28 days the activated sewage sludge nitrified more slowly than the other nitrogenous materials and produced a lower nitrate accumulation. At the lowest temperature level, however, it compared favorably with all of the materials employed, while at the highest temperature level it ultimately gave a nitrate accumulation comparable with that from the other materials, with the exception of sulfate of ammonia. In view of the fact that many of the favorable results reported for this material were obtained in northern states and in Canada, the data from these studies may be significant in indicating its value as an organic nitrogenous material in cold climates and in cool growing seasons. On the other hand the slow but steady response obtained at the highest temperature level would suggest a definite value for

activated sludge under midseason conditions. Its low nitrogen content, 5.1 per cent, is unfavorable for its utilization as a source of nitrogen in fertilizer mixtures, but its excellent mechanical condition and drying properties would suggest a definite value as a conditioner and auxiliary source of nitrogen. Inasmuch as this material consists very largely of dried and reversible organic colloids, the gradual rate of nitrification may be closely related to the colloidal property of the activated sewage sludge. Further investigations with this material are being conducted under field conditions, and the colloidal phase of the problem will be studied in more detail.

The amount of nitrogen added to these soils, as in Series I, was equivalent to 57.6 p.p.m. It will be noticed in table 5 that at the lowest temperatures, and up to the end of the 98-day period only one material, urea, had produced this amount of nitrate nitrogen. In the shaded soils in the main greenhouse, urea, tankage, sulfate of ammonia, and fish in the order named had produced practically this amount in 70 days. At the highest temperature level, after 42 days the added nitrogen was equaled in amount by the nitrate nitrogen produced from all the materials compared, except activated sewage sludge.

In comparison with soil-nitrification studies as reported by Fraps (29), and others, in which the maximum amount of nitrate formation took place within the first 4 weeks, the relatively slow rates of nitrification shown by this soil during the early weeks and the high nitrate accumulations in the later periods, are worthy of notice. Not only were the temperature levels employed lower than usually has been the case in such studies, but the bacterial population of this soil is comparatively low. The low content of total organic matter, and the porous drouthy conditions observed in the field, explain this deficiency. The work of Bonazzi (13) on the mechanism of nitrification would indicate that a carbon dioxide deficiency in the early stages may possibly have retarded the rate of nitrification.

With respect to H-ion concentration there was no observable correlation with the differences in mean temperatures. With the exception of sulfate of ammonia, which invariably decreased the pH values, the various materials gave only inconsistent and inconsequential differences in soil reaction.

Series III. The leaching of nitrates as influenced by various nitrogenous materials

The duration, the periods of sampling, the soil, and the analysis and rate of application of the fertilizer were the same in Series III as in Series I and II. Twelve glass percolation cylinders 7 inches in diameter by 14 inches in depth and slightly conical in shape, were used as containers. These were enclosed in a large wooden case 12 by 135 by 14 inches, as shown in figure 1.

To insure adequate drainage at the outlet and to prevent the loss of soil, 60 gm. of quartz gravel, the size of small marbles, was placed in the bottom of each cylinder and upon this was placed a layer of fine gravel. Each of the cylinders were then filled with 5500 gm. of sifted air-dry soil. The soil was moistened and leached six times between the dates of October 29 and November

26, to insure uniform drainage conditions. The leachings of November 26 contained an average of 4.3 mgm. of nitrate nitrogen. The top 3 inches—1000 gm.—of the soil was then removed from each cylinder and air-dried, after which it was thoroughly mixed with 5.5 gm. of 7-6-5 fertilizer and returned to the percolators. The fertilizer was mixed with the surface 3 inches of soil, rather than with the entire mass, to prevent mechanical loss from washing. The soil was then moistened by the addition of 200 cc. of distilled water and at 14-day intervals thereafter, the entire mass was leached with 825 cc. of distilled water added slowly to each cylinder. The volume of leachings obtained from each is shown in table 8. Calculated on the acre-inch basis for rainfall, and on the assumption that the weight of one acre of surface soil is 2 million pounds, these additions approximate a bi-weekly rainfall of $1\frac{1}{2}$ inches. Three days

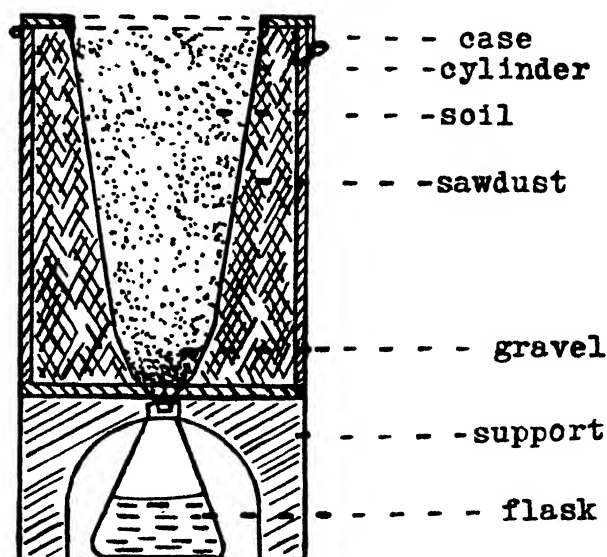


FIG. 1. DIAGRAMMATIC CROSS-SECTION OF CYLINDER AND CASE

after each addition of water the soil in each cylinder was cultivated to a depth of $\frac{1}{2}$ inch.

The application of 5.5 gm. of fertilizer to 5500 gm. of soil corresponds to a field application of 2000 pounds per acre of the fertilizer mixture, which contains the equivalent of 115.2 pounds of nitrogen. In each mixture all of the phosphorus was derived from 16 per cent acid phosphate and all of the potassium from potassium sulfate. Nitrate of soda, sulfate of ammonia, dried ground fish, and packing-house tankage were used singly and in combination as the sources of nitrogen. The various proportions of nitrogen carriers used for each cylinder are given in tables 6 and 7. In the fertilizers containing all four of these nitrogenous materials, the inorganic nitrogen was supplied in equal amounts by nitrate of soda and sulfate of ammonia, and the organic

TABLE 6
Nitrogen (as nitrate) leached at 14-day intervals with totals to date
 (Nitrogen added in fertilizer 316.6 mgm.)

NUMBER	SOURCE OF NITROGEN IN 7-6-5 FERTILIZER*	DECEMBER 31 14 DAYS		JANUARY 14 28 DAYS		JANUARY 28 42 DAYS		FEBRUARY 11 56 DAYS		FEBRUARY 25 70 DAYS		MARCH 11 84 DAYS		MARCH 25 98 DAYS		APRIL 8 112 DAYS	
		Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.	Leached mgm.	Total mgm.
1	Nitrate of soda	62.8	186.4	249.2	68.8	318.0	22.4	340.4	6.9	347.3	3.6	350.9	3.1	354.0	5.1	359.1	
2	Sulfate of ammonia	21.5	78.5	100.0	84.9	184.9	51.2	236.1	14.9	251.0	6.4	257.4	4.5	261.9	5.6	267.5	
3	Dried ground fish	15.9	15.9	68.8	84.7	68.6	153.3	51.8	205.1	21.1	226.2	16.8	243.0	10.0	253.0	10.8	263.8
4	Urea	25.1	25.1	105.0	130.1	76.2	206.3	40.5	246.8	9.1	255.9	5.2	261.1	4.7	265.8	6.7	272.5
5	Packing house tankage	21.8	21.8	76.1	97.9	71.8	169.7	39.2	208.9	20.5	229.4	12.8	242.2	8.1	250.3	10.8	261.1
6	$\frac{1}{2}$ nitrate; $\frac{1}{2}$ sulfate	79.3	79.3	127.6	206.9	74.3	281.2	30.0	311.2	9.8	321.0	5.5	326.5	4.2	330.7	6.2	336.9
7	40 per cent inorganic; 60 per cent organic	45.4	45.4	87.2	132.6	74.2	206.8	41.9	248.7	16.8	265.5	10.9	276.4	8.4	284.8	9.4	294.2
8	50 per cent inorganic; 50 per cent organic	55.7	55.7	106.4	162.1	54.4	216.5	42.7	259.2	15.8	275.0	11.6	286.6	6.1	292.7	8.2	300.9
9	60 per cent inorganic; 40 per cent organic	39.4	39.4	94.5	133.9	63.5	197.4	37.9	235.3	14.8	250.1	9.1	259.2	6.0	265.2	6.8	272.0
10	70 per cent inorganic; 30 per cent organic	43.1	43.1	127.6	170.7	67.4	238.1	37.0	275.1	12.6	287.7	7.5	295.2	7.3	302.5	6.9	309.4
11	80 per cent inorganic; 20 per cent organic	30.1	30.1	171.8	201.9	72.4	274.3	42.0	316.3	9.9	326.2	5.4	331.6	4.1	335.7	5.7	341.4
12	Check—0-6-5	15.4	15.4	19.7	35.1	6.9	42.0	8.4	50.4	3.6	54.0	5.1	59.1	3.1	62.2	5.3	67.5

* 5.5 gm. fertilizer per 5500 gm. soil.

nitrogen, in equal amounts by fish and tankage. Except for cylinder 4, in which urea was used as the sole source of nitrogen, these mixtures and rates of application are exact duplicates of those employed in the field-plot experiments.

Table 6 gives the milligrams of nitrate nitrogen leached at each 14-day interval with the total amounts to date. Table 7 gives the same information in terms of percentage of the added nitrogen leached to date at the end of each 14-day interval. Table 8 is included merely to show the drainage conditions which existed at each date of leaching throughout the total period of 112 days.

TABLE 7
Proportion of added nitrogen leached at 14-day intervals
(825 cc. distilled water—equivalent to 1.5 inches rainfall added every 14th day)

NUMBER	SOURCE OF NITROGEN IN 7-6-5 FERTILIZER	DECEMBER 31 14 DAYS	JANUARY 14 28 DAYS	JANUARY 28 42 DAYS	FEBRUARY 11 56 DAYS	FEBRUARY 25 70 DAYS	MARCH 11 84 DAYS	MARCH 25 98 DAYS	APRIL 8 112 DAYS
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	Nitrate of soda	15.0	67.6	87.2	91.6	92.6	92.2	92.2	92.1
2	Sulphate of ammonia	1.9	20.5	45.1	58.6	62.2	62.6	63.1	63.2
3	Dried ground fish	0.2	15.7	35.2	48.9	54.4	58.1	60.3	62.0
4	Urea	3.1	30.0	51.9	62.0	63.8	63.8	64.3	64.8
5	Packing house tankage	2.0	19.8	40.3	50.1	55.4	57.9	59.4	61.1
6	$\frac{1}{2}$ nitrate; $\frac{1}{2}$ sulfate	20.2	54.3	75.6	82.4	84.3	84.5	84.8	85.1
7	40 per cent inorganic; 60 per cent organic	9.5	30.8	52.1	62.6	66.8	68.6	70.3	71.6
8	50 per cent inorganic; 50 per cent organic	12.7	40.1	55.1	66.1	69.8	71.8	72.8	73.7
9	60 per cent inorganic; 40 per cent organic	7.6	31.2	49.1	58.4	61.9	63.2	64.1	64.6
10	70 per cent inorganic; 30 per cent organic	8.7	42.8	61.9	71.0	73.8	74.6	75.9	76.4
11	80 per cent inorganic; 20 per cent organic	4.6	52.7	73.4	84.0	86.0	86.1	86.4	86.5
12	Check 0-6-5 (subtracted in each case for the corresponding period)								

* 5.5 gm. fertilizer per 5500 gm. soil.

The original soil contained 0.041 per cent of total nitrogen, or 2.255 gm. in each cylinder, to which was added 0.3166 gm. in the fertilizer mixture. From table 6 it may be observed that after subtracting the amount leached from the checks, the total amount of nitrate-nitrogen recovered after 112 days was in no case equal to that added in the fertilizer. Other investigators have shown that soluble salts of phosphorus and potassium stimulate the nitrification of the nitrogen of the soil. A comparison of the 4.3 mgm. of nitrate-nitrogen per cylinder, obtained from the last leaching prior to the addition of the fertilizer, with the amounts shown for cylinder 12 at the 14- and 28-day

periods, indicates a marked stimulation of nitrification by the acid phosphate and potassium sulfate. A part of this stimulation must be ascribed, however, to the thorough aëration of the portion of soil removed for mixing with the fertilizer.

It must be recognized that not all of the nitrogen leached from any one of the cylinders came from the fertilizer. In the two series just described, there were a number of instances in which the nitrate accumulation exceeded the amount of added nitrogen plus the nitrate accumulation in the soils receiving phosphate and potash only. This indicates that the nitrification of the soil nitrogen was stimulated by the nitrogenous materials as well as by the phosphorus and potassium salts. Even though the nitrate leached from any of the cylinders

TABLE 8
Total volume of leachings at various time intervals

NUM- BER	TREATMENT	14 DAYS	28 DAYS	42 DAYS	56 DAYS	70 DAYS	84 DAYS	98 DAYS	112 DAYS
		cc	cc	cc.	cc	cc	cc	cc.	cc.
1	Nitrate of soda	295	705	1,195	1,730	2,172	2,517	2,700	3,068
2	Sulfate of ammonia	420	985	1,433	1,988	2,455	2,871	3,179	3,574
3	Dried ground fish	392	962	1,467	2,047	2,449	2,891	3,176	3,523
4	Urea	379	949	1,324	1,919	2,329	2,692	2,970	3,325
5	Packing house tankage	345	910	1,355	1,892	2,327	2,737	2,997	3,342
6	$\frac{1}{2}$ nitrate; $\frac{1}{2}$ sulfate	410	1,008	1,522	2,079	2,557	2,980	3,290	3,698
7	40 per cent inorganic; 60 per cent organic	445	1,013	1,476	2,063	2,546	2,976	3,299	3,652
8	50 per cent inorganic; 50 per cent organic	397	947	1,352	1,928	2,332	2,762	3,009	3,376
9	60 per cent inorganic; 40 per cent organic	394	939	1,297	1,762	2,126	2,481	2,729	3,029
10	70 per cent inorganic; 30 per cent organic	365	901	1,273	1,763	2,143	2,507	2,783	3,088
11	80 per cent inorganic; 20 per cent organic	445	975	1,337	1,862	2,262	2,687	2,989	3,333
12	No nitrogen	358	914	1,292	1,857	2,222	2,670	2,890	3,220

receiving nitrogen did not exceed that leached from the check cylinder by the amount added, it is evident that a part of the soil nitrogen was leached and that a part of the added nitrogen was utilized by soil microorganisms. A clearer comparison of the various materials is obtained, however, by subtracting the nitrate leached from the check cylinder at each time period, from that leached from each of the other cylinders, and by expressing the net amounts as percentages of the nitrogen added in the fertilizer.

Table 7 shows in general that the rapidity of leaching and the amount of nitrates lost in the drainage water vary directly with the proportion of the nitrogen supplied by nitrate of soda. Conversely the greater the proportion of organic ammoniates, the slower was the rate of leaching and the smaller the total amounts lost during the 112-day period. From cylinders 1, 6, and 11,

to which the nitrogen was added in the forms of nitrate of soda, of the 50-50 ratio nitrate of soda and sulfate of ammonia, and of the 80-20 ratio respectively, more than 50 per cent of the added nitrogen was leached at the first two periods, i.e. by 3 inches of rainfall. By the third period the equivalent of 50 per cent or more of the added nitrogen had been leached from the soils receiving nitrogen as urea, and as the 40-60, 50-50, and 70-30 inorganic-organic ratios. By the fourth period the sulfate of ammonia, the tankage, and the 60-40 ratio had leached to the same extent. The cylinder receiving fish, however, did not lose nitrogen to this extent until the fifth period, or after 70 days had elapsed and the equivalent of a total of $7\frac{1}{2}$ inches of rainfall had been added.

The treatments ranked as follows with respect to the total amount of nitrate leached: 1. nitrate of soda; 2. the 80-20 ratio; 3. $\frac{1}{2}$ nitrate of soda— $\frac{1}{2}$ sulfate of ammonia; 4. the 70-30 ratio; 5. the 50-50 ratio; 6. the 40-60 ratio; 7. urea; 8. the 60-40 ratio; 9. sulfate of ammonia; 10. fish; 11. tankage. * These may also be considered the relative rates of availability.

Cylinder 9, which received its nitrogen from the 60-40 ratio is evidently out of line, although no satisfactory explanation has been found. It may be due in part to a greater evaporation from the surface of the soil and hence to a slightly smaller total volume of leachings, as shown in table 8. For the first three periods, however, the volume of leachings from this cylinder was greater than from some of the others. The growth of oats, planted in these cylinders after the 112-day period, seems to indicate that an appreciable amount of the fertilizer nitrogen added to this cylinder remains in the soil. In fact, for all the cylinders the growth of this crop is inversely proportional to the amount of nitrate leached.

At the start of this experiment no analyses were made to determine the amounts of ammonia-nitrogen which may have leached from the cylinders receiving sulfate of ammonia. It is not probable that any appreciable amount of ammonia-nitrogen was leached as such. The following considerations would support this belief: (a) the fact that the soil was slightly acid, pH 6.4; (b) the fertilizer was added to the surface 3 inches only; (c) 14 days elapsed between the moistening of the soil and the date of the first leaching; (d) the actual amount and the proportion of *nitrate* leached at the first period from the cylinder receiving nitrate of soda were relatively small. The fact that nitrates leach much more rapidly than ammonia, especially in acid soils, has long been recognized. Fraps (28) has shown that 500-gm. portions of soil, to which ammonium chloride had been added to the extent of 51.7 p.p.m. of ammonia nitrogen, when leached immediately, retained from 61 to 98.5 per cent of the ammonia added. For cylinder 2, to which all of the nitrogen added was in the form of sulfate of ammonia, the 316.6 mgm. of nitrogen added to 5500 gm. of soil is equivalent to 57.5 p.p.m. of ammonia nitrogen. In view of the afore-stated considerations and conditions of this experiment, it is improbable that any appreciable amount of nitrogen was lost in the leachings in the ammonia form.

Considering from another angle, this question for cylinder 2, the 5.5 gm. of fertilizer added contained 1.493 gm. sulfate of ammonia. Adding the approximate 1200 cc. of capillary moisture held by the soil in each cylinder, to the 825 cc. of water used to leach the soil, gives a total of approximately 2 liters of water present at each leaching. These amounts of water and sulfate of ammonia would give approximately a $\frac{1}{12}$ *N* solution. Wolkoff (82) found that on adding 4 cc. of a $\frac{1}{12}$ *N* ammonium sulfate solution to 20 gm. of Sassafras medium sandy loam, the freezing point depression was lowered 47 per cent because of absorption. Garretson (32) in studying the soils of Java, added sulfate of ammonia in amounts equal to 1 per cent of the weight of the soil. He found that after nitrification, about 81.5 per cent of the added nitrogen could be leached as nitrate; whereas before nitrification, from one to two per cent only of the added nitrogen was leached by the same procedure. From the foregoing it seems permissible to assume that the leaching of nitrogen in the form of ammonia did not appreciably influence the total loss of nitrogen in the drainage water.

The rapid leaching of nitrates during the early periods from cylinder 4, in which urea was used entirely as the source of nitrogen, is in complete agreement with the pot experiments of Series I and II. The relatively small total amount of nitrate leached from this cylinder during the entire period, is also in agreement with the data of the preceding experiments, in that the total nitrate accumulation from this material was in many instances less than from sulfate of ammonia, fish, or tankage.

There are several factors which influence the leaching of nitrogen applied to soils in commercial fertilizers. Briefly, they are: the chemical form in which the nitrogen is applied, the organic matter and nitrogen content of the soil, the microbiological flora, the mechanical composition, the moisture content, and the soil temperature, all of which influence the rate of nitrate formation. The influence of these factors was demonstrated in the pot cultures of Series I and II. In this Series the influence of various combinations of nitrogenous materials on the rate of nitrification is apparent. Since all useful nitrogenous fertilizer materials produce appreciable amounts of nitrate nitrogen in the soil within the growing season in which the application is made, the question resolves itself to that of a balance between the *rate* of nitrification and the soil and climatic factors which favor or inhibit rapid leaching. The combinations of nitrogenous materials employed in this experiment indicate that within certain limits the rate of nitrate formation may be controlled by the judicious mixing of various materials.

It is well known that, because of climatic and soil temperature conditions, early vegetable crops can best be produced on sandy soils. Such soils are always relatively deficient in clay and silt, i.e. in colloids and the materials from which colloids may readily be formed. Although the soil selected for these leaching experiments may present a rather extreme type of these soils, in that it contains nearly 89 per cent total sands and only 11 per cent silt and

clay, it affords an excellent medium for the comparison of nitrogenous materials. Furthermore, considerable areas of this soil exist, and may be cropped successfully when the sources of nitrogen are adequately balanced, and the method and time of application are adapted to the existing conditions.

There is a definite and well defined nitrogen problem with these soils. Their mechanical composition, the summer drouth, and the cropping system to which they are best adapted, all make it impractical to attempt to build up high organic matter and total nitrogen contents. The cash crops require appreciable quantities of nitrate nitrogen early in the season, in order to force growth against the influence of unfavorable soil and air temperatures. At such times the soil temperature is usually too low to promote rapid nitrification of many of the commercial organic materials used in fertilizers. Consequently some nitrate nitrogen must be included. Small local areas in the Sassafras soils and extensive areas of the Norfolk soils, have subsoils containing even less silt and clay than the surface soil. The present experiment has shown that soils deficient in colloidal material have a relatively small capacity for the absorption of nitrates.

At the experimental field at Snow Hill, Maryland, there was a total rainfall of 18.2 inches during the growing season for the early potato crop, from March 26 to June 30, 1924. In addition, the date of planting had been considerably delayed by excessive rainfall prior to March 26. Both on May 24 and June 9, more than 3 inches of rain was measured by the rain-gauge within 24-hour periods. On May 18 nearly 2 inches of rain fell, and on March 29, April 6, and May 21, 1 inch or more of rainfall was recorded. These heavy precipitations undoubtedly leach a considerable proportion of the nitrate nitrogen present at the time from the surface soil to the subsoil. Unless the subsoil has an appreciably higher silt and clay content than the surface soil and if its capillary contact is broken by a layer of relatively coarse sand, it is doubtful whether much of the nitrate-nitrogen leached is again brought to the surface. Especially in these tidewater regions, lying from 5 to 20 feet above mean sea level, and in which the permanent water table is found at a depth of 2 to 4 feet, the recovery of nitrates once leached from the surface soil is evidently quite small.

The duration of the leaching experiments was 112 days, during which time the equivalent of 12 inches of rainfall had been added to each cylinder. In the field during the 1924 season, this amount of precipitation had occurred within 62 days after the early potato crop had been planted. Table 7 shows that, from cylinders 1, 6, and 11, which received 100, 50, and 40 per cent respectively of the added nitrogen as nitrate of soda, the equivalent of 92 to 85 per cent of the fertilizer nitrogen had been leached. In fact 6 inches, or one-half the amount of rainfall, in 6 days less time, leached 91.6, 82.4, and 84.0 per cent respectively of the amount of nitrogen added to these cylinders. From this it is evident that for these soils when the amount of nitrate present is in excess of the feeding-power of the crop plus the absorptive capacity of the soil, the

amount of nitrate leached depends entirely on the characteristics of the sub-soil and the distribution and amount of rainfall. The malnutrition of the early potato crop, so frequently observed on these soils, may thus be explained by the fact that too great a portion of the fertilizer nitrogen was supplied as nitrates or readily nitrifiable materials.

Aside from the growing of leguminous green manure crops whenever practicable, the nitrogen problem for these soils resolves itself into that of using a fertilizer that will yield sufficient nitrate nitrogen to produce the early growth desired, but which at no time will give a large accumulation of nitrate to be lost in the drainage water. To obtain this result all of the type materials compared in these experiments can be employed profitably, but the first essential is a clear understanding of the soil conditions influencing the rate at which each material produces nitrate nitrogen.

SUMMARY AND CONCLUSIONS

1. The optimum soil-moisture content for nitrate production from various nitrogenous materials applied in a 7-6-5 complete fertilizer, was found between 50 and 60 per cent of the water-holding capacity for Norfolk sandy loam soil.

2. There was a larger nitrate accumulation from urea at 70 per cent of the water-holding capacity of the soil than from sulfate of ammonia, dried ground fish, or packing house tankage.

3. After the first period of rapid nitrification, the fluctuations of nitrate nitrogen in this soil appear to be the result of the activity of soil microorganisms, rather than of differences in soil-moisture, temperature, or reaction.

4. Up to 20 to 30°C., which was the highest temperature range employed, nitrate production from sulfate of ammonia, urea, dried ground fish, packing-house tankage, and activated sewage-sludge increased directly with the temperature.

5. At all temperatures, urea showed the most rapid rate of nitrification; at all except the lowest temperature level, sulfate of ammonia gave the highest ultimate accumulation of nitrate.

6. When both rate of nitrate production and rate of nitrate accumulation in this soil are considered, the materials employed rank in the following order: nitrate of soda, urea, sulfate of ammonia, dried ground fish, packing-house tankage, and activated sewage sludge.

7. The leaching of nitrates from Norfolk sandy loam soil, following an application of a 7-6-5 fertilizer equivalent to 2000 pounds per acre, varied directly with the proportion of the nitrogen supplied by nitrate of soda in the mixture.

8. When used as the sole source of nitrogen in this analysis ratio, there was little difference in the total amounts of nitrates leached from sulfate of ammonia, urea, dried ground fish or packing-house tankage; although more nitrate-nitrogen leached during the early periods from the cylinder receiving nitrogen from urea.

REFERENCES

- (1) ALBRECHT, W. A. 1919 Nitrate production in soil as affected by the crop and cultivation. *Missouri Agr. Exp. Sta. Bul.* 163: 67-68.
- (2) ALBRECHT, W. A. 1922 Nitrate accumulation under straw mulch. *Soil Sci.* 14: 299-305.
- (3) ALBRECHT, W. A. 1924 Soil studies at the Missouri Station. *Missouri Agr. Exp. Sta. Bul.* 210.
- (4) ALLISON, F. E., BRAHAM, J. M., AND MCMURTEY, J. E., JR. 1924 Field experiments with atmospheric nitrogen fertilizer. *U. S. Dept. Agr. Bul.* 1180.

- (5) ALLISON, F. E., VLIET, E. B., SKINNER, J. J., AND REID, F. R. 1924 Greenhouse experiments with atmospheric nitrogen fertilizers and related compounds. *Jour. Agr. Res.* 28: 971-976.
- (6) APPLEMAN, C. O. 1918 Physiological basis for the preparation of potatoes for seed. *Md. Agr. Exp. Sta. Bul.* 212.
- (7) BARTHEL, C. 1920 Nitrate formation in soil. *Svensk Kem. Tidskr.* 32: 173-183. (Abs. in *Chem. Abs.* 15: 1053. 1921.)
- (8) BARTHEL, C., AND BENGSTON, N. 1920 The nitrification of stable manure nitrogen in soil. *Meddel. Centralanst. Försöksv. Jordbruksområdet (Sweden)* 211: 18. (Abs. in *Exp. Sta. Rec.* 45: 423.)
- (9) BIZZELL, J. A. 1922 Disappearance of nitrates in soil under timothy. *Jour. Amer. Soc. Agron.* 14: 320-326.
- (10) BLAIR, A. W. 1922 Agricultural and commercial values of nitrogenous plant foods. *Jour. Amer. Soc. Agron.* 14: 162-167.
- (11) BLAIR, A. W., AND PRINCE, A. L. 1922 Variation of nitrate nitrogen and pH values of soils from the nitrogen availability plots. *Soil Sci.* 14: 1-19.
- (12) BLAIR, A. W., AND PRINCE, A. L. 1924 Influence of varying ratios and phosphoric acid and potash on crop yield and nitrogen recovery. *Soil Sci.* 17: 327-331.
- (13) BONAZZI, A. 1921 On nitrification. IV. The carbon and nitrogen relations of the nitrite ferment. *Jour. Bact.* 6: 479-499.
- (14) BONAZZI, A. 1923 On nitrification. V. The mechanism of ammonia oxidation. *Jour. Bact.* 8: 343-363.
- (15) BRIGGS, L. J., ET AL. 1904 The Centrifugal Method of Soil Analysis. *U. S. Dept. Agr. Bur. Soils Bul.* 24
- (16) BRENCHEY, W. E., AND RICHARDS, E. H. 1920 The fertilizing value of sewage sludge. *Jour. Soc. Chem. Indus.* 39: 177 T-181 T.
- (17) BROWN, H. D. 1920 Report on the fertilizer value of activated sludge. *Ontario Bd. Health Ann. Rpt.* 39: 130-155.
- (18) BROWN, P. E., AND GOWDA, R. N. 1924 The effect of certain fertilizers on nitrification. *Jour. Amer. Soc. Agron.* 16: 137-146.
- (19) CARPENTER, P. H., AND BOSE, A. K. 1921 Ammonia, nitrate and carbon dioxide formation in relation to the best mechanical soil condition. *Indian Tea Assoc., Sci. Dept. Quart. Jour* 3: 103-120.
- (20) CHARDON PALACIOS, C. E. 1921 Preliminary studies on ammonification and nitrification in Puerto Rico soils. *Rev. Agr. Puerto Rico* 5: p. 37-46. (Abs. in *Exp. Sta. Rec.* 44: 814)
- (21) CHEVALIER, C. 1923 Utilization of synthetic urea as a nitrogenous fertilizer. *Prog. Agr. et Vitic. (Ed. l'Est-Centre)* 44: 257-260. (Abs. in *Exp. Sta. Rec.* 50: 424.)
- (22) CLARKE, G. R. 1924 Soil acidity and its relation to the production of nitrate and ammonia in woodland soil. *Oxford Forestry Mem.* 2: 27.
- (23) COLEMAN, D. A. 1917 The influence of sodium nitrate upon nitrogen transformations in soils with special reference to its availability and that of other nitrogenous manures. *Soil Sci.* 4: 345-432.
- (24) COLEMAN, L. C. 1907 Researches on nitrification. *Cent. Bakt.* 2: 401, 484.
- (25) DENISON, I. A. 1922 The nature of certain aluminum salts in the soil and their influence on ammonification and nitrification. *Soil Sci.* 13: 81-106.
- (26) EHRENBURG, F., AND SCHULTZE, K. 1916 Contributions to the explanation of the manganese question. *Jour. Landw.* 64: 37-129.
- (27) ERDMAN, L. W. 1923 The effect of gypsum on Iowa soils. *Soil Sci.* 15: 137-155.
- (28) FRAPS, G. S. 1920 Nitrification in Texas Soils. *Texas Agr. Exp. Sta. Bul.* 259.
- (29) FRAPS, G. S. 1921 Relation of soil nitrogen, nitrification and ammonification to pot experiments. *Texas Agr. Exp. Sta. Bul.* 283.

- (30) FRED, E. B., AND GRAUL, E. J. 1916 Some factors that influence nitrate formation in acid soil. *Soil Sci.* 1: 317-338.
- (31) GARDER, T., AND HAGUM, O. 1919 Studies on nitrification and H-ion concentration. *Bergens Mus. Aarbok. Naturv. Raekke*, 1919-20: (1), 1-31. (Abs. in *Exp. Sta. Rec.* 50: 722.)
- (32) GARRETSON, F. C. 1921 An investigation on nitrification and denitrification in tropical soils. *Arch. Suikerindus. Nederland. Indië* 29: 1397-1460; 1461-1530 (Abs. in *Exp. Sta. Rec.* 47: 214.)
- (33) GEILMAN, W. 1922 The washing out of ammonia and nitrate nitrogen from sand soils. *Jour. Landw.* 70: 259-266.
- (34) GOWDA, R. N. 1924 Oxidation of ammonia and nitrites by micro-organisms under different conditions. *Soil Sci.* 17: 57-64.
- (35) GOWDA, R. N. 1924 Nitrates and nitrification in field soils. *Soil Sci.* 17: 333-342.
- (36) GREAVES, J. E. 1922 Influence of salts on soil bacteria. *Bot. Gaz.* 73: 161-180.
- (37) GREAVES, J. E., AND CARTER, E. G. 1925 Influence of moisture on the bacterial activities in the soil. *Soil Sci.* 19: 361-387.
- (38) HALL, THOMAS D. 1923 Nitrification in some South African soils. *Soil Sci.* 18: 219-235.
- (39) HARPER, HORACE J. 1924 The accurate determination of nitrates in soils—phenol-di-sulfonic acid method. *Jour. Indus. Engin. Chem.* 16: 180-183.
- (40) HARRIS, F. S., AND BUTT, N. I. 1917 Effect of irrigation water and manure on the nitrates and total soluble salts of the soil. *Jour. Agr. Res.* 8: 333-359.
- (41) HARRISON, W. H. 1923 The movement of nitrates in soil and subsoil. *Agr. Res. Inst., Pusa, Sci. Rpts.* 1922-23: 29-31.
- (42) HASELHOFF, E. 1922 Experiments with Rehmsdorf organic nitrogenous fertilizer. *Ztschr. Pflanzenernähr. u. Düngung.* 1. *Wirtschaft-Prakt.*: 510-519. (Abs. in *Exp. Sta. Rec.* 50: 623.)
- (43) HASSELHOFF, E., LIEHR, O., AND FLUHRER, K. 1922 Experiments with nitrogenous fertilizer. *Landw. Vers. Sta.* 100: 35-58.
- (43a) HILGARD, E. W. 1912 *Soils*. New York.
- (44) HUTCHINSON, C. M. 1922 Soil studies at the Pusa Research Institute. *Agr. Res. Inst., Pusa, Sci. Rpts.* 1921-22: 41-42.
- (45) JACOB, K. D., ALLISON, F. E., AND BRAHAM, J. M. 1924 Chemical and biological studies with cyanamid and some of its transformation products. *Jour. Agr. Res.* 28: 27-69.
- (46) JOHNSON, T. C. 1923 Four years results with potatoes at the Virginia Truck Experiment Station. *Armour & Co. Farmers Almanac* 1923.
- (47) KADISH, V. H. 1924 Activated sludge, its manufacture and fertilizing value. *Amcr. Fert.* 60: 78-91.
- (48) KUYPER, J. 1923 The nitrogenous fertilizers ammonium sulphate, nitrate, and urea. *Arch. Suikerindus. Nederland. Indië* 31: 21-25. (Abs. in *Exp. Sta. Rec.* 49: 20)
- (49) LEMMERMANN, O., AND ECKL, K. 1923 Investigation on the action of ammonium bicarbonate in comparison with ammonium sulphate and sodium nitrate in, different methods of application. *Ztschr. Pflanzenernähr. u. Düngung.* 2. *Wirtschaft-Prakt.*: 98-109. (Abs. in *Exp. Sta. Rec.* 51: 120.)
- (50) LEMMERMAN, O., AND WICHERS, L. 1920 The periodic influence of the time of year on the process of nitrification. *Centbl. Bakt.* (II) 50: 33-43.
- (51) LIPMAN, C. B., AND BURGESS, P. S. 1916 Ammonifiability versus nitrifiability as a test for the relative availability of nitrogenous fertilizers. *Soil Sci.* 3: 63-75.
- (52) LIPMAN, J. G., AND BLAIR, A. W. 1918 Twenty years work on the availability of nitrogen in nitrate of soda, ammonium sulfate, dried blood and farm manures. *Soil Sci.* 5: 1-9.

- (53) LIPMAN, J. G., AND BLAIR, A. W. 1918 The value of nitrate of soda in crop production. *N. J. Agr. Exp. Sta. Bul.* 323.
- (54) LITTAUER, F. 1924 Decomposition of urea in soil, *Ztschr. Pflanzenernähr. u. Düngung. Wiss.*: 165-179. (Abs. in *Exp. Sta. Rec.* 51: 818.)
- (55) LOHNIS, F. 1923 Influence of time of year on nitrate formation in soil. *Centbl. Bakt.* (II) 58: 207-211.
- (56) MARTIN, WILLIAM H. 1923 Feed balanced fertilizer ration for best potato yield returns. *N. J. Agr.* 4: (12): 6.
- (57) MEEK, C. S., AND LIPMAN, C. B. 1922 The relation of the reaction and of salt content of the medium on nitrifying bacteria. *Jour. Gen. Phys.* 5: 195-204.
- (58) MIYAKE, K., AND SOMA, S. 1922 Further studies on the nature of nitrification. *Jour. Biochem.* 1: 123-127.
- (59) MUNTER, F. 1920 Investigations on chemical and bacteriological transformation in soil. *Landw. Jahrb.* 55: 62-138.
- (60) MUNTER, F. 1923 The action of physiologically acid and alkaline nitrogen salts. *Ztschr. Pflanzenernähr. u. Düngung* 2: *Wirtschaft-Prakt.*: 497-516. (Abs. in *Exp. Sta. Rec.* 51: 121.)
- (61) MURPHY, H. F. 1924 Nitrification experiments on soils of the red prairies. *Jour. Amer. Soc. Agron.* 16: 130-136.
- (62) Nebraska Agricultural Experiment Station 1923 Soil fertility studies at the Nebraska Station. *Ann. Rpt.* 1923.
- (63) PANGANIBAN, ELIAS H. 1925 Temperature as a factor in nitrogen changes in the soil. *Jour. Amer. Soc. Agron.* 17: 1-31.
- (64) PRESCOTT, J. A. 1923 The volatilization of the ammonia of ammonium sulphate and soil reaction. *Bul. Union Agr. Égypte* 151: 121-128.
- (65) ROBINSON, C. S., WINTER, O. B., AND MILLER, F. G. 1921 Studies on the availability of organic nitrogenous compounds. *Jour. Indus. Engin. Chem.* 13: 933-936.
- (66) SACHS, W. H., AND AUSTIN, R. H. 1922 The effect of cultivation during the growing season on moisture content and formation of nitrates in the soil. *Ark. Agr. Exp. Sta. Bul.* 181: 41-42.
- (67) SCHERMERHORN, LYMAN G. 1924 Potash can change shape of sweet potatoes. *N. J. Agr.* 6: (4) 6-7.
- (68) SCHNEIDEWIND, W., MEYER, D., AND MUNTER, F. 1920. Nitrogen experiments. *Landw. Jahrb.* 55: 1-20.
- (69) SHEDD, O. M. 1919 Effect of oxidation of sulphur in soils on the solubility of rock phosphate and on nitrification. *Jour. Agr. Res.* 18: 329-345.
- (70) SCHONBRUNN, B. 1922 Progress of nitrification with time, with special reference to the periodic influence of time of year. *Centbl. Bakt.* (II) 56: 545-565.
- (71) SMITH, R. S. 1920 Some effects of potassium salts on soils. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 35: 571-605.
- (72) STARKEY, R. L. 1924 Some observations on the decomposition of organic matter in soils. *Soil Sci.* 17: 293-314.
- (73) STEVENSON, R. E. 1920 Nitrification in acid soils. *Iowa Agr. Exp. Sta. Res. Bul.* 58: 331-349.
- (74) TRUFFAUT, G., AND BEZSSONOFF, N. 1924 The most favorable form of nitrogen for major crops. *Compt. Rend. Acad. Sci.* 178: 723-25.
- (75) TRUFFAUT, G., AND BEZSSONOFF, N. 1924 On the form of nitrogen most favorable to higher plants. *Sci. Sol.* (Truffant) 3: 1-19.
- (76) United States Department of Agriculture Year Book 1921 Cost data for farm products, p. 824.
- (77) VAN KENIERNUM, W. 1922 Fertilizer Experiments with different new nitrogenous fertilizers. *Mitt. Deut. Landw. Gesell.* 37: 654-655.

- (78) WAKSMAN, S. A. 1923 Influence of nitrogen fertilization upon microbiological activities of the soil. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1922: 359-361.
- (79) WAKSMAN, S. A. 1923 Microbiological analysis of soils as an index for soil fertility: V. Methods for the study of nitrification. *Soil Sci.* 15: 241-260.
- (80) WHITING, A. L., AND SCHOONOVER, W. R. 1920 Nitrate production in field soils in Illinois. *Ill. Agr. Exp. Sta. Bul.* 225: 21-63.
- (81) WILSON, B. D. 1923 The effect of plants on the concentration of drainage water from the Cornell lysimeters. *Soil Sci.* 16: 427-432.
- (82) WOLKOFF, M. I. 1917 Adsorption of ammonium sulfate by soils and quartz sand. *Soil Sci.* 3: 561-564.
- (83) WOODS, CHAS. D. 1918 Potatoes grown on Aroostook farm on fertilizers containing ammonia (nitrogen) in different forms. *M. Agr. Exp. Sta. Bul.* 269: 37-39.

FERTILITY OF A SOIL AS RELATED TO THE FORMS OF ITS IRON AND MANGANESE

P. H. BREWER AND R. H. CARR

*Purdue University*¹

Received for publication May 5, 1926

For a long time it has been known that many soils fail to give a good crop response, even after adequate applications of fertilizers containing nitrogen, phosphorus, and potassium, and after the correction of the acidity through the addition of limestone. The plants growing in these soils often give evidence of soil toxicity, such as by lack of suitable growth, yellowing of the leaves, and white stripes developing in the leaves. Evidence accumulating in recent years leads one to believe that the cause of the trouble is closely associated with the forms of the iron and manganese contained in the soil. It is well known that iron is essential to plant growth and it is believed that small amounts of manganese are also desirable and necessary (11) for good plant growth, but their relationship to soil toxicity and plant disease is a matter of more recent development.

EVIDENCE OF SIMILAR TOXICITY IN THE HAWAIIAN SOILS

The Hawaiian soils which are unusually high in iron and manganese have been investigated by Kelley (9), Johnson (8), McGeorge (10), and others, because of their toxicity to sugar cane and pineapple. A difference of opinion exists as to the real cause of pineapple chlorosis. McGeorge (11) believes the chlorosis found in the manganese soils "is due to a greater accumulation of lime indirectly caused by the presence of manganese in excessive amounts in the soil." Gile (7) found in Porto Rico that with heavy applications of stable manure to manganese soil containing about 30 per cent of lime it was possible to obtain a normal growth of pineapples, and Kelley in Hawaii found that this heavy manure treatment made it possible to obtain a good growth of pineapples on manganese soils.

FORMS OF IRON AND MANGANESE IN CERTAIN INDIANA SOILS

The presence of iron and manganese in forms which cause toxicity has been noted in many different soils in Indiana as well as in other states, but especially is this true of the experimental fields (11) near Bedford and Scottsburg, Indiana. It was thought worth while to study this latter area and to call atten-

¹Division of Agricultural Chemistry.

tion to the similarity of the treatments which have been helpful here and in the Hawaiian soils. The Scottsburg soil is what is known as "yellow clay" and responds well to manure treatment (as shown in plots 14 and 15, table 1),

TABLE 1

Limestone requirement, crop yields, and potassium thiocyanate color changes of the Scottsburg soils

PLOT NUMBER	LIME REQUIRED BY KCNS pounds	COLOR* OF KCNS SOLUTION ON STANDING				TREATMENT	AVERAGE CROP YIELDS 1906-1922		
		1 hour	36 hours	60 hours	100 hours		Corn	Wheat	Clover
							bushels	bushels	pounds
1†	2,500	DR	DR	DR	DR	T	23.9	7.1	189
2†	2,300	DR	DR	DR	DR	T, NPK	32.2	12.7	364
3	1,300	R	R	R	R	L, NPK	36.2	13.6	468
4	1,400	R	R	R	R	L	22.9	6.9	288
5	900	R	LR	LR	LR	L, NPK‡	35.1	12.2	671
6	700	R	LR	LR	LR	L, NPK§	35.9	13.7	622
7	200	P	G	G	VG	Treatment changed 1920			
8	600	LR	P	VLG	VLG	L	27.0	8.4	457
9	100	P	C	G	VG	L, AP	35.0	12.4	655
10	2,000	DR	DR	DR	DR	O	20.6	6.9	274
11	1,800	DR	DR	DR	DR	AP	32.9	11.1	522
12	700	R	LP	C	G	L, RP	40.0	11.5	769
13	1,200	R	LR	P	VLR	L	28.7	8.0	509
14	1,200	R	P	G	VG	L, RP, M	53.5	17.5	1552
15	1,400	R	LP	LG	G	L, M	52.7	16.8	1344
16	900	LR	LG	G	VG	L	28.0	8.0	571
17	1,200	R	LR	G	VG	L, PK	38.0	14.0	854
18	1,100	R	LR	G	VG	L, NPK	39.4	16.9	713
19	1,100	R	LP	G	VG	L	27.6	8.0	475
20	900	R	LG	G	VG	L, NPK	40.3	16.1	815
21	1,000	R	LG	G	VG	L, NP	41.5	14.7	855
22	1,000	R	LP	G	VG	L	28.8	8.8	584

* P, pink, DR, dark red; LR, light red; C, colorless; G, green; VG, very green.

† Timothy instead of clover in rotation.

‡ Half of P from bone and half from acid phosphate.

§ P from steamed bone.

L—2 tons limestone per acre—1911.

M—10 tons manure on corn until 1919 lowered to 5 tons in 1920.

AP—150 pounds 16 per cent acid phosphate on wheat (began 1915); 150 pounds 16 per cent acid phosphate on corn.

RP—1 ton rock phosphate per acre 1905 also in 1911.

NPK—300 pounds 2-8-4 on wheat and additional; 300 pounds on corn beginning 1915.

but not well to complete fertilizer. As in the Hawaiian soils, the iron and manganese toxicity is not lessened much by liming, although the toxic aluminum has been largely overcome by this treatment as is shown by the lime

requirement. Other soils, such as that at North Vernon, with low organic content but with no appreciable amount of manganese in the dioxide form, usually respond well to treatment of commercial fertilizers, and good crop yields are obtained.

Table 1 shows the treatment and crop yield of the Scottsburg plots from 1906 to 1923. In addition, the limestone requirement by the quantitative potassium thiocyanate method (3) is given, as well as the effect of the presence of manganese dioxide upon the fading out of the red color of ferric thiocyanate.

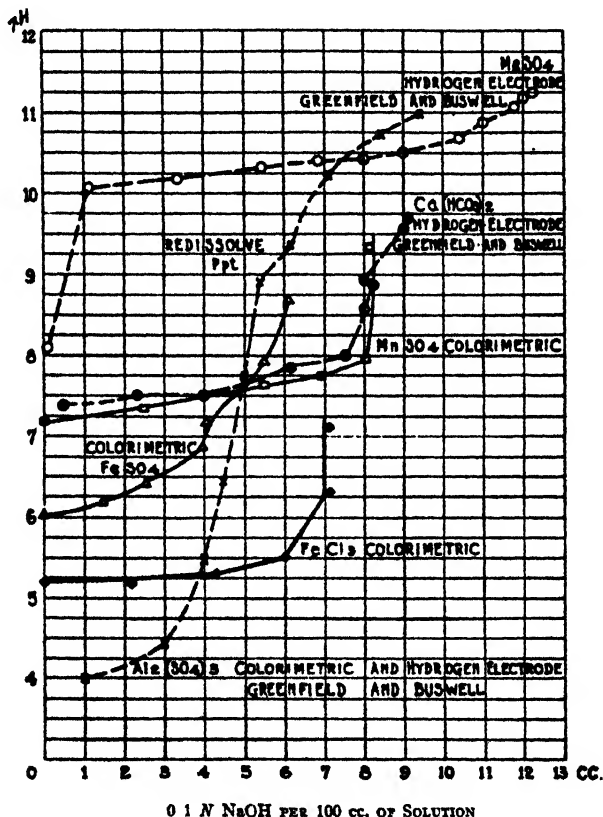


FIG. 1. PRECIPITATION OF ALUMINUM, FERRIC AND FERROUS IRON, MANGANESE AND MAGNESIUM AS HYDROXIDES AND CALCIUM BICARBONATE AS CALCIUM CARBONATE

(*Indus. and Engin. Chem.* 15: 634)

It will be noted from table 1 that the crop increase due to the use of manure in plots 14 and 15 was considerable, and that no combination of commercial fertilizers produced similar yields. The addition of rock phosphate made little improvement over manure in the crop yield in plot 14. Hence it is evident that the organic matter was able to correct, to a considerable extent,

the toxicity which the application of complete commercial fertilizers did not greatly alter. One important reason for the great crop increases in plots 14 and 15 is believed to be the reduction, by the rotting manure, of some of the manganese dioxide and some of the ferric iron to the ferrous form, in which condition iron remains in solution and available to the plant when the reaction of the soil is less acid than pH 5.5 as previously shown by the writers (fig. 1). It is believed by Johnson (6) that the improved pineapple growth reported in the Hawaiian soils from the addition of manure was due to the availability of the iron reduced from the ferric to the ferrous condition by the manure. It will be noted from figure 1 that ferrous iron is not easily precipitated as a hydroxide and seems to be available to plants even when the soil is about pH 8.

METHODS OF DETERMINING MANGANESE DIOXIDE AND FERROUS IRON IN SOIL

In this investigation one of the methods used to determine manganese dioxide quantitatively in the soil was a modified Bunsen method in which hydrochloric acid is added to the soil, the liberated chlorine is distilled into a

TABLE 2
Showing the weight of manganese dioxide added to five-gram samples of soil, and the amounts recovered

SOIL NUMBER	MnO ₂ ADDED .	MnO ₂ RECOVERED	DIFFERENCE
	gm.	gm.	
1	0.1531	0.1521	0.0010
2	0.1392	0.1347	0.0045
3	0.1516	0.1436	0.0080
4	0.1392	0.1384	0.0008
5	0.1531	0.1476	0.0055
6	0.1516	0.1436	0.0080

potassium iodide solution, and the free iodine thus formed is treated with standard sodium thiosulfate. Since organic matter in the soil seriously interferes with chlorine liberation, different compounds were tried to get rid of this interference as much as possible. The addition of 0.2 gm. of mercuric oxide to 5 gm. of soil was found to be fairly successful. This causes some kind of a combination between the mercury and organic material when boiling with the hydrochloric acid. The mercury-organic compound could be seen moving about in the form of "balls" in the boiling mass. In order to test the accuracy of this method, a known amount of manganese dioxide was added to each of a series of different soil types having an organic content from three to seven per cent (manganese dioxide was not found in soils high in organic matter) and then an attempt was made to recover it. The data thus obtained are given in table 2.

FERROUS AMMONIUM SULFATE METHOD OF DETERMINING MANGANESE DIOXIDE IN SOIL

Manganese dioxide in soil was determined more satisfactorily by use of a ferrous ammonium sulfate method as follows:

Five grams of soil and 100 ml. of a 5 per cent solution of ferrous ammonium sulfate to which had been added concentrated sulfuric acid at the rate of 6 ml. to the liter were placed in a tall shaker bottle of about 200 ml. capacity. The soil and solution were thoroughly mixed and the bottle was then placed in boiling water for 30 minutes. The soil and solution were mixed by shaking several times during the digesting period. After the digestion had been completed the bottle was placed in an upright position and allowed to remain until the soil had settled. Twenty-five-milliliter samples were pipetted off into 250-ml. Erlenmeyer flasks, and 50 ml. of water, 25 ml. of concentrated nitric acid, and about $\frac{1}{2}$ gm. of sodium bismuthate were added and the contents boiled slowly for about ten minutes. If the red color of the sodium permanganate disappeared, more sodium bismuthate was added and the contents were boiled again. This process was repeated until the red color remained after 5 minutes of gentle boiling. A few milliliters of 5 per cent sodium nitrate solution was added to reduce any manganese dioxide that may have formed during the boiling. The flask was next cooled to room temperature and about 0.4 gm. of sodium bismuthate was again added. The flask and contents were allowed to stand for 15 to 20 minutes so that all the manganese would be oxidized to permanganate. The contents of the flask were filtered through an asbestos filter, washed, and the filtrate was titrated at once with standard sodium arsenite.

DETERMINATION OF SOLUBLE MANGANESE (OTHER THAN MANGANESE DIOXIDE) AND THE FERROUS IRON IN A SOIL

It has been shown by Mitschek (13) that 0.27 *N*, H_2SO_4 does not dissolve manganese dioxide. This has been verified by the writers as shown by data in figure 2. The easily soluble manganese in the soil (other than manganese dioxide) and the ferrous iron were dissolved by heating 10 gm. of 100-mesh soil with 200 ml. of 0.2 *N* H_2SO_4 in boiling water for 30 minutes. When cool, aliquots were taken for the determination of manganese as given above (leaving out the ferrous ammonium sulfate). The ferrous iron was determined by titrating an aliquot with 0.1 *N* potassium dichromate using diphenolamine as the indicator. Data obtained by this method checked fairly well with the Bergeim (2) method of obtaining ferrous iron in feces. The results obtained by the various methods just described are given in table 2.

GREEN COLOR OF POTASSIUM THIOCYANATE SOIL SOLUTION AS AN INDICATOR OF MANGANESE DIOXIDE

In a previous article (4) the writers called attention to the green color when determining the limestone requirement by the quantitative potassium thiocyanate method (3) and attempted an explanation of the cause of the red color fading out when the Comber (5) qualitative method for soil acidity was used on soils. A method to obviate the fading out of the red color in this process has been suggested by Emerson (6). He concludes that the fading out is due to the presence of soluble phosphates and has developed a solution for testing

soils in which the interfering phosphate is first precipitated. This method, however, was used on the soils noted in table 1, as well as on many other soils containing manganese dioxide, and, as when the Comber solution was used, the red was found to fade out or become less red depending on the amount of manganese dioxide. This would indicate some other cause for this fading out.

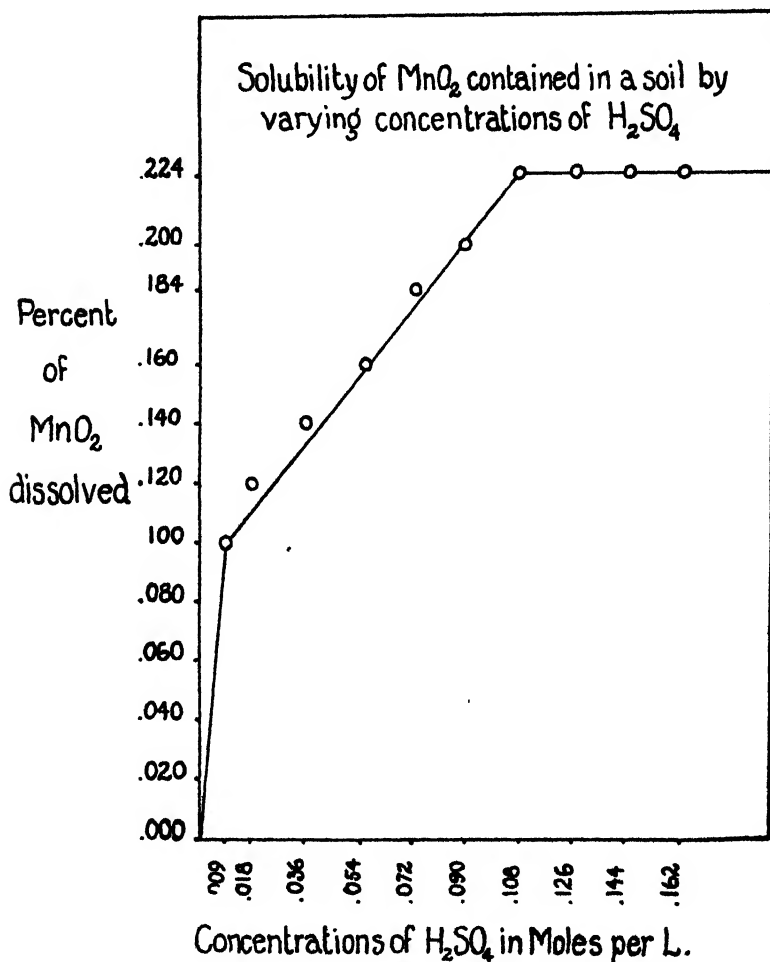


FIG. 2

NATURE OF THE GREEN MANGANESE SOLUTION

McGeorge (11) has taken exception to a statement of the writers relative to the pH at which manganese is precipitated as a hydroxide from salts of manganese and from the potassium thiocyanate compound in alcohol. The difference of opinion seems to be a question of solution vs. colloidal suspension

of the manganese compound. The writers believe, in contradistinction to McGeorge, that the green color of the KCNS solution is not due to a definite chemical compound, but largely to a colloidal suspension containing manganese which causes a scattering of light, as shown by the strong Tyndal cone effect observed in the green liquid. Anderegg and Lutz (1) believe that this colloidal suspension is produced by the mutual effect of oppositely charged colloids, such as the absorption of the positive ferric iron by the negative manganese

TABLE 3
Amounts of ferrous iron and manganese dioxide obtained by the various methods

PLOT NUMBER	MnO ₂ BY BUNSEN'S METHOD	MnO ₂ † BY Fe(NH ₄) ₂ (SO ₄) ₂ PLUS H ₂ SO ₄ (A)	MnO ₂ * BY H ₂ SO ₄ (B)	DIFFERENCE IN MnO ₂ (A - B)	Fe IN H ₂ SO ₄ ‡
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.065	0.200	0.116	0.084	0.106
2	0.068	0.180	0.120	0.060	0.106
3	0.055	0.174	0.084	0.090	
4	0.055	0.160	0.084	0.076	
5	0.054	0.188	0.140	0.048	0.112
6	0.053	0.200	0.136	0.064	0.112
7	0.087	0.208	0.142	0.062	0.106
8	0.060	0.204	0.128	0.076	0.106
9	0.065	0.244	0.180	0.064	0.095
10	0.060	0.232	0.180	0.052	0.106
11	0.075	0.224	0.136	0.086	0.106
12	0.073	0.240	0.194	0.056	0.127
13	0.056	0.244	0.184	0.060	0.127
14	0.026	0.236	0.200	0.036	0.191
15	0.030	0.268	0.244	0.044	0.191
16	0.066	0.284	0.152	0.132	0.095
17	0.076	0.296	0.188	0.108	0.106
18	0.064	0.326	0.184	0.136	0.106
19	0.065	0.320	0.176	0.144	0.085
20	0.074	0.328	0.184	0.144	0.095
21	0.068	0.356	0.232	0.124	0.106
22	0.077	0.324	0.204	0.120	0.085

* Manganese other than manganese dioxide.

† Total manganese.

‡ Data obtained by C. H. Keipper, unpublished.

dioxide or by such other negative colloids as Fuller's earth or Putman's clay. Analyses of the green KCNS compound in alcohol show the presence of large amounts of manganese. The pH had been previously determined by the colorimetric method, but has been repeated electrometrically recently with other soil types, by the use of the quinhydrone electrode. The results show that the liquids with the green suspension have a pH of about 6, and that all were still somewhat green at pH 8.5, after the additions of successive increments of 0.1 N alcoholic potassium hydroxide.

CAUSES OF THE FADING OUT OF THE RED COLOR OF FERRIC THIOCYANATE

It is well known that a basic solution of soluble phosphate will combine with and displace the iron of the ferric thiocyanate ($\text{Fe}(\text{CNS})_3$) compound, but it is believed by the writers that there is usually no appreciable amount of soluble phosphate present in a wet or moist soil at the same time with easily soluble ferric iron available to form ferric thiocyanate. If such were the case they would soon combine in the soil to form iron phosphate.

It is possible to take out the red color of $\text{Fe}(\text{CNS})_3$ from a soil solution in various ways, as, in some cases, by adding water; soil containing MnO_2 ; MnO_2 in pure form; soluble bases or phosphates, borates, or arsenates; or many organic acids such as oxalic, tartaric, citric, and malic. On the other hand, the red color may be restored by adding a ferric salt in excess of the MnO_2 , or by lowering the pH to dissolve more iron.

DISCUSSION

It will be noted from table 1 that in plots 14 and 15 there is less MnO_2 in the soil, and that more of the iron is in the ferrous form, than in any of the other plots. It will be noted also in table 1 that in plots 1 to 4, and 10 to 11, to which there had not been enough lime or phosphate added to precipitate the iron as a hydroxide or phosphate, the red did not fade out, but in all the others the red color disappeared in about 36 hours, the solution became colorless, and a very green color developed in about 100 hours. It is thus evident that the shade of red (or green) obtained by the Comber method in testing manganese soils is determined not only by the amount of base or phosphate compound present but also by the extent to which the ferric iron is in excess of the manganese dioxide in the soil sample. As one of the writers (3) has previously pointed out, the use of the KCNS color standard alone as a guide to the lime requirement of a soil, is, therefore, not reliable.

SUMMARY

A study of the different plots of the Scottsburg experiment field indicates that a toxic substance is present in the soil and that the iron present is mostly in the ferric condition. The conditions in the soil which cause the oxidation of the manganese to the dioxide form also change most of the ferrous iron to ferric, in which form it is not available to plants when the acidity is about pH 6.0. This condition is counteracted to a considerable extent by the reducing action of manure but not by commercial fertilizers. The manure added to the soils studied did not greatly change the percentages of iron and manganese present in the soil, but their less oxidized forms seem to be more favorable to plant growth. This change is believed to have been the main cause for an annual increase of about one-third in the corn and wheat yields and an approximate doubling of the clover yield.

The presence of manganese dioxide can usually be recognized by the forma-

tion of a green color when the soil is tested by the quantitative potassium thiocyanate method for acidity. The manganese dioxide often present in soil will cause a fading out of the red color of $\text{Fe}(\text{CNS})_3$, if there is less available iron than manganese (MnO_2) present. The KCNS color standard method thus may indicate a basic soil, whereas in reality the soil is acid.

REFERENCES

- (1) ANDEREGG, F. O., AND LUTZ, R. P. 1925 Colloidal complications in the thiocyanate method of estimating soil acidity. *Jour. Indus. and Engin. Chem.* 17: 1153.
- (2) BERGEIN, OLAF 1924 The estimation of intestinal reductions. *Jour. Biol. Chem.* 62: 45.
- (3) CARR, R. H. 1921 Measuring soil toxicity, acidity and basicity. *Jour. Indus. and Engin. Chem.* 13: 931.
- (4) CARR, R. H., AND BREWER, P. H. 1923 Manganese aluminum and iron ratio as related to soil toxicity. *Jour. Indus. and Engin. Chem.* 15: 634.
- (5) COMBER, N. M. 1920 A qualitative test for sour soils. *Jour. Agr. Sci. (England)* 10: 420-422.
- (6) EMERSON, PAUL 1923 A suggested laboratory and field test for soil acidity. *Jour. Amer. Soc. Agron.* 15: 495-499.
- (7) GILE, P. L. 1911 Relation of calcareous soils to pineapple chlorosis. *P. R. Agr. Exp. Sta. Bul.* 11.
- (8) JOHNSON, M. O. 1924 Manganese chlorosis of pineapples; its cause and control. *Hawaii Agr. Exp. Sta. Bul.* 52.
- (9) KELLEY, W. P. 1912 The function and distribution of manganese in plants and soils. *Hawaii Agr. Exp. Sta. Bul.* 26.
- (10) McGEORGE, W. T. 1923 Chlorosis of pineapple plants grown in manganiferous soils. *Soil Sci.* 16: 269-74.
- (11) McGEORGE, W. T. 1924 Acidity of highly basic soils. *Soil Sci.* 16: 195-206.
- (12) McHARGUE, J. S. 1922 The rôle of manganese in plants. *Jour. Amer. Chem. Soc.* 44: 1592-1597.
- (13) MITSCHKE, H. 1925 Determination of manganese in highly alloyed special steels and ferrous alloys containing cobalt. *Chem. Ztg.* 49: 25-27.
- (14) Report of the Scottsburg Experiment Field. 1906-1922. *Ind. Agr. Exp. Sta.*

OUTGO OF CALCIUM, MAGNESIUM, NITRATES AND SULFATES FROM HIGH-CALCIC AND HIGH-MAGNESIC LIMES INCOR- PORATED IN TWO SOIL ZONES

W. H. MacINTIRE¹

University of Tennessee Agricultural Experiment Station

Received for publication May 17, 1926

Although soil literature is replete with comparisons between calcium and magnesium in their more active forms, very little attention has been paid to the effects produced by joint additions of these two elements in the proportions approximating those found in magnesian limes. Insufficient consideration has been accorded the possible antagonistic effects between calcium and magnesium *after* their joint absorption from near-equal additions. It has been generally assumed that preponderant additions of either calcium or magnesium result in reciprocal liberations in the soil; but it has been demonstrated that such an interchange may not follow when either caustic or carbonate forms are incorporated throughout the surface soil. When measured by the rain-fall leachings *under humid conditions* (6, 7), liberation not only failed to occur in certain surface soils, but calcium and magnesium incorporations were reciprocally repressive upon native soil complexes of magnesium and calcium. The reverse was found in the case of subsoil (6). There the slowly moving nitrate- and sulfate-impregnated solutions did effect an interchange, which was influenced by concentration of the neutral salts of one element, irrespective of an accumulation of the carbonate of the same element in the subsoil through deposition from its bicarbonate carried by soil leachings (6). Hence, not only may the soil solution be altered in its proportion of dissolved Calcium and Magnesium as a result of increase of the element added in excess, but the change in proportion may be further accentuated through repressed solubility of the soil's content of the other element.

Although high-calcic lime, precipitated CaCO_3 , and limestone may decrease the outgo of magnesium from the surface soil when the additions are mixed throughout the full depth of soil, and although MgO , precipitated MgCO_3 , and magnesite may effect a similar decrease in calcium outgo, an increase in the outgo of both calcium and magnesium may or may not, come from full-depth incorporations of dolomite (6, 7) at different rates. One Ca:Mg proportion obtained in the leachings when the dolomite underwent complete

¹ The results were obtained by Mr. H. Stanford, Mr. T. D. Harden, and Dr. R. M. Barrette all of whom were formerly employed as assistants through an endowment from the National Lime Association, with equipment donated by the American Limestone Company.

TABLE 1

Total calcium, magnesium, and calcium-magnesium leached during 4 years from 2,000-pounds CaO equivalents (3,570 pounds CaCO₃) of hydrated lime, burnt dolomite, and mixture of separately calcined CaO and MgO incorporated in the surface zone of a loam soil—terms of CaCO₃-equivalence per 2,000,000 pounds of moisture free soil

TREATMENT	FIRST YEAR						SECOND YEAR						THIRD YEAR						FOURTH YEAR				TOTAL FOR 4 YEAR PERIOD		4-YEAR INCREASE IN OUTGO OVER CONTROLS	
	May to September	September to January	January to March	March to May	Total	lbs.	May to December	December to February	February to May	Total	lbs.	May to September	September to February	February to May	Total	lbs.	May to December	December to May	Total	lbs.	Total	lbs.	Actual	On basis of addition	per cent	
Calcium as CaCO ₃ ⇌																										
Controls.....	128	136	53	40	357	108	43	37	188	69	81	27	177	126	50	176	898	
Ca(OH) ₂	240	201	55	47	543	138	43	39	220	79	82	27	188	149	41	190	1,141	243	6.8	243	6.8	243	6.8	243	6.8	
CaO-MgO*.....	214	204	52	36	506	113	41	34	188	88	83	24	195	136	41	177	1,066	168	7.9†	168	7.9†	168	7.9†	168	7.9†	
CaO-MgO†.....	150	223	53	46	472	122	43	36	201	75	73	27	175	128	36	164	1,012	114	5.4‡	114	5.4‡	114	5.4‡	114	5.4‡	
Magnesium as CaCO ₃ ⇌																										
Controls.....	34	39	18	20	111	34	20	20	74	31	32	14	77	43	31	74	336	
Ca(OH) ₂	59	52	16	17	152	38	21	21	80	24	26	15	65	43	24	67	364	28	...	28	...	28	...	28	...	
CaO-MgO*.....	53	56	16	17	138	37	20	21	78	32	39	18	89	59	35	94	399	63	4.3†	63	4.3†	63	4.3†	63	4.3†	
CaO-MgO†.....	37	56	17	20	130	41	21	21	83	24	32	18	74	60	31	91	378	42	2.9‡	42	2.9‡	42	2.9‡	42	2.9‡	

disintegration, and another when there was a persistence of undisintegrated particles (6, p. 323-328; 7, p. 450, 451). Again, the ratio of calcium outgo to magnesium outgo from an incorporation of dolomite in the upper zone will vary decidedly from that obtained from the same incorporation in the lower zone, when the dolomite is of sufficient fineness to insure near-complete disintegration (2). It therefore becomes desirable to determine what relationship obtains for Ca:Mg outgo when calcium and magnesium are added jointly in caustic form and in amounts which are completely, or almost completely, fixed by the surface soil (4) in its upper and lower zones.

In comparing the pulverulent and more active calcium and magnesium materials, it is not permissible to base the comparison solely upon those properties of the alkali-earth materials which are characteristic of them prior to their absorption by the soil. Thorough incorporation of economic additions of caustic forms in a similar loam soil resulted in rapid and practically complete absorption of the base within 10 days (1, 9), under favorable moisture conditions. It is important to remember, therefore, that after a short period—often only a few days—the properties of the absorption complexes become of paramount importance. Those of calcium differ from those of magnesium in their resistance to hydrolysis, and such differences may be reflected in the reactions which take place after economic additions are “fixed” in the soil.

OBJECTIVES

The present contribution is based upon a 4-year study of the influences exerted by three equivalent caustic limes, subsequent to the period of a few days required for their complete absorption. Natural leachings of Calcium, Magnesium, Ca-Mg, nitrates, and sulfates were obtained by means of an outdoor lysimeter equipment in comparing: 1. High-calcic and high-magnesian burnt limes; 2. Burnt dolomite and equivalent mixtures of CaO and MgO, derived from separately calcined marble and magnesite; 3. Surface-zone and subsurface-zone incorporations in their effects upon (1) and (2); 4. Initial and subsequent availability of absorbed calcium and Ca-Mg, as influenced by *diminishing residues* and by *aging*.

EXPERIMENTAL

Two tanks containing a fertile, carbonate-free, brown, acid loam were compared with six tanks containing the same soil and additions of the three caustic limes. These tanks were included in a group previously described and illustrated (3). Plant growth was excluded and the soils were undisturbed during the 4-year period. The soil contained 0.2617 per cent of CaO and 0.3439 per cent of MgO when placed. These total percentages were equivalent to 9340 pounds and 17,072 pounds of CaCO_3 , respectively, on the basis of 2,000,000 pounds of moisture-free soil. Each addition was incorporated only in the upper zone, or half, of the surface soil, in comparison

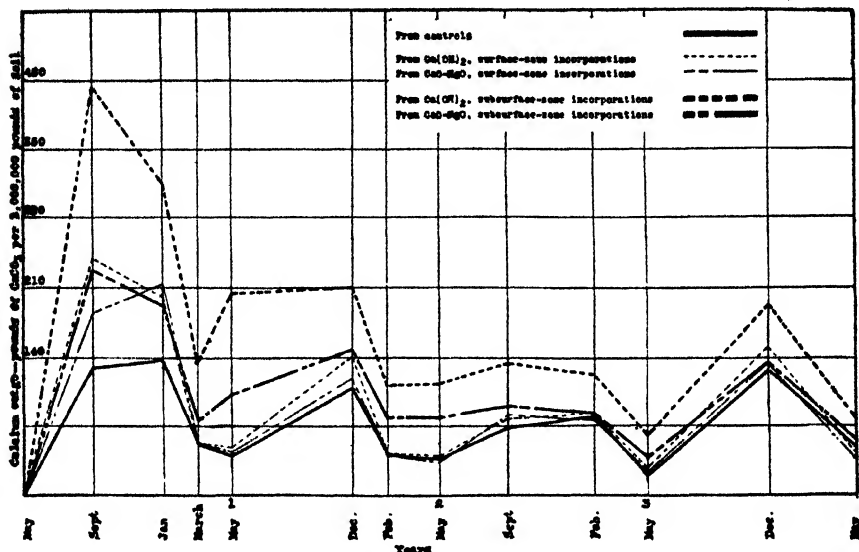


FIG. 1. PROGRESSIVE CALCIUM OUTGO, AS POUNDS OF CaCO_3 -EQUIVALENT PER 2,000,000 POUNDS OF SOIL, FROM CONTROLS, SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF $\text{Ca}(\text{OH})_2$ AND CaO-MgO DURING A 4-YEAR PERIOD

All incorporations at the equivalent rate of 3750 pounds of CaCO_3 (2000 pounds CaO)

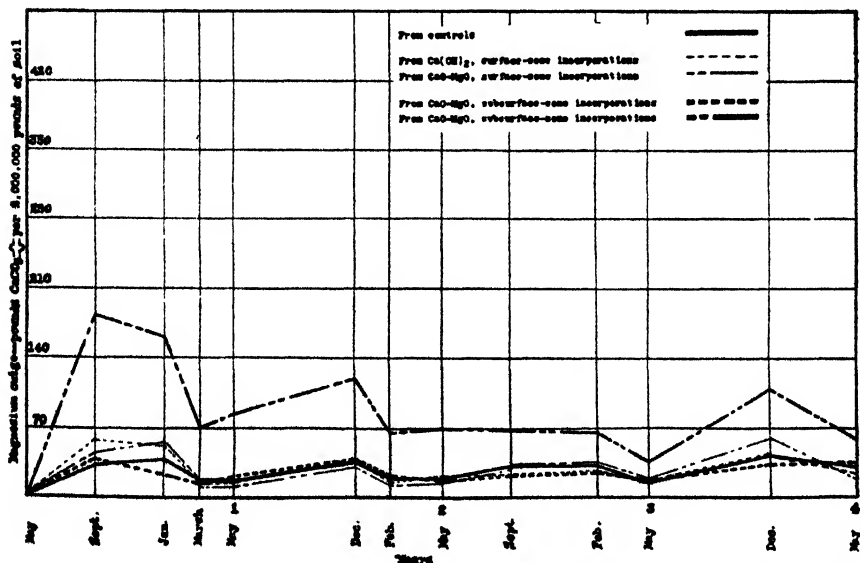


FIG. 2. PROGRESSIVE MAGNESIUM OUTGO, AS POUNDS OF CaCO_3 -EQUIVALENT PER 2,000,000 POUNDS OF SOIL, FROM CONTROLS, SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF $\text{Ca}(\text{OH})_2$ AND CaO-MgO DURING A 4-YEAR PERIOD

All incorporations at the equivalent rate of 3750 pounds of CaCO_3 (2000 pounds CaO)

with the same incorporation in the lower zone without addition to the upper zone. The incorporations were all equivalent to 3570 pounds of CaCO_3 (2000 pounds CaO) per 2,000,000 pounds of soil, moisture-free basis when the full depth, or both zones, are considered. On the same basis, all leachings of calcium, magnesium, and Ca-Mg are expressed as pounds of CaCO_3 . The enhancements in outgo, over the average from the controls, are also expressed as pounds and as per cent of the addition of calcium, magnesium, or Ca-Mg .

The high-calcic lime was practically free of MgO , whereas the two high-magnesian materials contained 46.53 per cent CaO and 32.85 per cent MgO . In each CaO-MgO incorporation an equivalence of 2000 pounds of CaO —3570

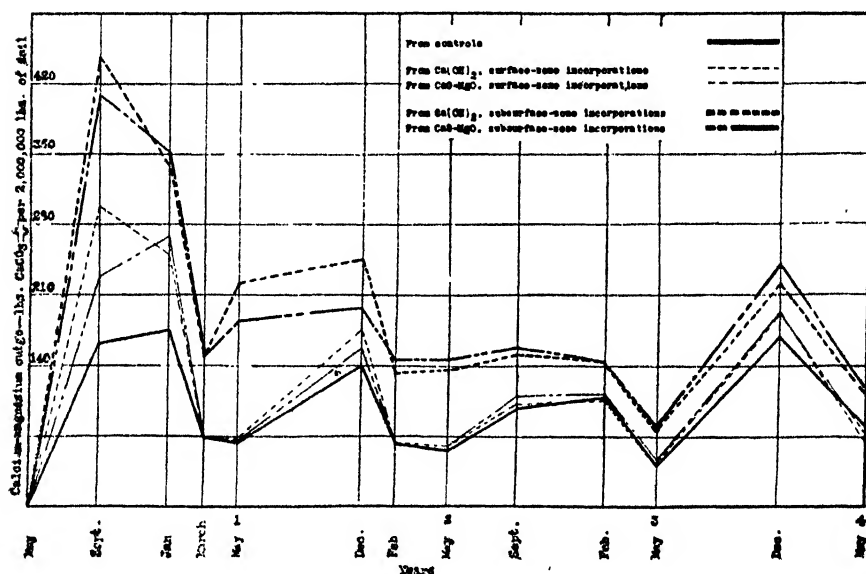


FIG. 3. PROGRESSIVE CALCIUM-MAGNESIUM OUTGO, AS POUNDS OF CaCO_3 -EQUIVALENT PER 2,000,000 POUNDS OF SOIL, FROM CONTROLS, SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF Ca(OH)_2 AND CaO-MgO DURING A 4-YEAR PERIOD

All incorporations at the equivalent rate of 3750 pounds of CaCO_3 (2000 pounds CaO)

pounds of CaCO_3 —was accounted for jointly by 1184.8 pounds and 815.2 pounds for CaO and MgO , respectively. On the basis of chemical equivalence the ratio of CaO:MgO was therefore 1.453 to 1.

DISCUSSION

Calcium, magnesium, and Ca-Mg outgo

Surface-zone incorporations. The outgo of calcium, magnesium and Ca-Mg from controls and from each surface-zone incorporation is given in table 1 and in figures 1, 2, and 3. The leachings from this upper-zone series were

subject, of course, to stoppage by the untreated lower zone. In considering the relationship of Ca:Mg outgo from the several incorporations, it is well to remember that the outgo of calcium from the controls was always greater than that of magnesium. During the first year the Ca:Mg outgo was at the ratio of 3.2:1, and about 2.4:1 as an average during the last 3 years.

The results show maximum outgo from the controls and maximum increase in outgo of both calcium and magnesium from all incorporations during the first year. Thereafter the differences, if any, between controls and treatments were small. The high-calcic lime yielded 102 pounds more of CaCO_3 than the average outgo from the two high magnesian limes for the 4-year period and one-half of this increase was accounted for during the first year. The CaO content of the $\text{Ca}(\text{OH})_2$ was 1.69 times that of the magnesian limes, whereas the total increase in calcium outgo from the hydrated lime was 1.72 times that as an average from the CaO-MgO incorporations.

The magnesium outgo from $\text{Ca}(\text{OH})_2$ was somewhat greater than that from the controls. This was the result of the first year's outgo and may readily be accounted for by assuming that an interchange of calcium for magnesium took place during the first year of maximum generation of nitrates and sulfates, while these salts were passing through the lower zone to which no $\text{Ca}(\text{OH})_2$ was added. The average of the magnesium losses from the two magnesian limes was less than that from $\text{Ca}(\text{OH})_2$ and only 23 pounds in excess of that from the control during the first year. Thereafter it was generally comparable with that from the controls. However, the average enhancement in calcium outgo from the magnesian limes during the first year was 132 pounds as against 186 pounds from the hydrated lime incorporation which carried 1.69 times more calcium. A similar relationship obtains for the 4-year totals, and it is thus apparent that either less magnesium than calcium passed from the upper zone or more magnesium than calcium was stopped by the lower, untreated zone. This point will be considered later in connection with the parallel data from the subsurface-zone incorporations. The maximum acceleration in Ca-Mg outgo came from the $\text{Ca}(\text{OH})_2$, and amounted to only 7.6 per cent of the addition. The results from the two magnesian limes did not vary greatly, but the outgo from the separately calcined mixture was generally less than that from the equivalent calcined dolomite.

Subsurface-zone incorporations. The results from these incorporations, devoid of any diminishing effect exerted by a lower, untreated zone, are given in table 2 and shown graphically in figures 1, 2, and 3. Disregarding capillary rise of soluble calcium salts from the lower to the upper zone, the rate of treatment in the lower zone might well be regarded as twice that where the full depth of soil is considered. That is, lower-depth incorporations were actually on the basis of 3570 pounds CaCO_3 -equivalence (2000 pounds CaO) per 1,000,000 pounds of soil, since there was no supplementary influence of an underlying zone. The increase in calcium outgo from the $\text{Ca}(\text{OH})_2$ was 707 pounds for the first year, or 60 per cent of that for the 4-year period.

TABLE 2

Total calcium, magnesium, and calcium-magnesium leached during 4 years from 2,000-pound CaO equivalents (3,570 pounds CaCO_3) of hydrated lime, burnt dolomite, and mixtures of separately calcined CaO and MgO incorporated in the subsurface zone of a loam—terms of CaCO_3 -equivalence per 2,000,000 pounds of moisture-free soil

TREATMENT	FIRST YEAR					SECOND YEAR					THIRD YEAR					FOURTH YEAR					4-YEAR INCREASE IN OUTGO OVER CONTROLS	
	May to September	September to January	January to March	March to May	Total	May to December	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total	lbs.	Total for 4-YEAR PERIOD	lbs.	Actual	On basis of addition	Per cent
Calcium as CaCO ₃ ⇌																						
Controls.....	128	136	53	40	357	108	43	37	188	69	81	27	177	126	50	176	898
Ca(OH) ₂	412	315	135	202	1,064	210	113	115	438	133	121	60	314	188	77	265	2,081	1,183	33.1			
CaO-MgO*.....	254	193	83	104	634	157	78	80	315	98	77	41	216	138	50	188	1,353	455	21.5†			
CaO-MgO†.....	199	188	73	98	558	138	79	75	292	84	83	39	206	123	57	180	1,236	338	16.0‡			
Magnesium as CaCO ₃ ⇌																						
Controls.....	34	39	18	20	111	34	20	20	74	31	32	14	77	43	31	74	336
Ca(OH) ₂	37	25	14	20	96	36	21	21	78	18	23	17	58	33	36	69	301	—35			
CaO-MgO*.....	186	155	68	81	490	118	63	68	249	72	62	42	176	126	55	181	1,096	760	52.2†			
CaO-MgO†.....	177	168	76	85	506	117	71	69	257	64	67	39	170	92	63	155	1,088	752	51.7‡			

Calcium-magnesium as $\text{CaCO}_3 \rightleftharpoons$

Controls.....	162	175	71	60	468	142	63	57	262	100	113	41	254	169	81	250	1,234
$\text{Ca}(\text{OH})_2$	449	340	149	222	1,160	246	134	136	516	151	144	77	372	221	113	334	2,382	1,148	32.1
CaO-MgO^*	440	348	151	185	1,124	275	141	148	564	170	139	83	392	264	105	369	2,449	1,215	34 0†§
CaO-MgO^\dagger	376	356	149	183	1,064	255	150	144	549	148	150	78	376	215	120	335	2,324	1,090	30.5†§

* Calcined dolomite 46.53 per cent CaO , 32.85 per cent MgO —Supplying 1184.8 pounds CaO and 815.2 pounds $\text{CaO} \rightleftharpoons$ respectively.

† Mixtures of CaO and MgO from separately calcined marble and magnesite.

‡ Based on CaO content of high magnesian lime.

§ Based on MgO content of calcined CaO and calcined MgO mixture.

There was a decided drop to a 250-pound excess above the controls for the second year, with further progressive diminution in outgo for the third and fourth years. The full increase for the 4-year period was only 33.1 per cent of the addition. The 1183-pound enhancement of CaO outgo from the $\text{Ca}(\text{OH})_2$ incorporations for the 4-year period was 2.98 times the average enhancement

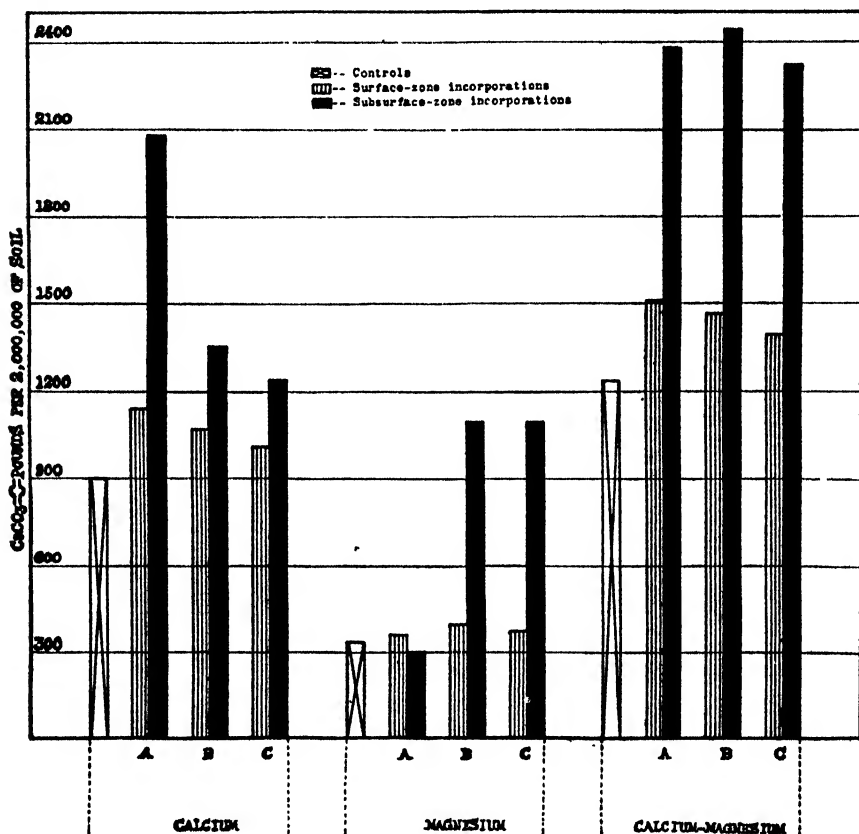


FIG. 4. FOUR-YEAR OUTGO OF CALCIUM, MAGNESIUM, AND Ca-Mg IN TERMS OF CaCO_3 FROM SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF HYDRATED LIME, BURNED DOLOMITE, AND CaO-MgO MIXTURES AT THE RATE OF 3570 POUNDS OF CaCO_3 (2000 POUNDS CaO) PER 2,000,000 POUNDS OF SOIL

A, hydrated lime; B, burnt dolomite; C, separately calcined CaO and MgO

of 397 pounds from the two magnesian limes, although the corresponding ratio of CaO added was approximately 1.69:1. The freshly absorbed non-carbonate complexes furnishes the increase in calcium outgo from the $\text{Ca}(\text{OH})_2$; but, in the case of the CaO-MgO incorporations, the absorbed calcium was not sufficient in amount and distribution to inhibit hydrolysis of some of the absorbed magnesium.

The same order of annual outgo—maximum for the first year, with progressive decrease thereafter—obtains in the calcium results from the two magnesian limes. Each annual outgo of calcium from the separately calcined oxides was less than the corresponding one from the chemically equivalent burned dolomite. It is possible that variations in critical temperatures of calcination may account for these differences.

The magnesium content of the leachings from the high-calcic lime was less than that from the untreated soil during three of the four years, and the 4-year total was less. This indicates a repression of the solubility of native magnesium, in accord with previous observations in parallel studies where there was no opportunity for interference of underlying zones (6, 7). The total magnesium losses from the two types of magnesian lime were in accord, and, in general, those of the several collections and annual period were comparable. Although the average enhancements in outgo of calcium and magnesium from the surface-zone incorporations of the two magnesian limes showed a Ca:Mg ratio of 2.66:1, the lower-zone incorporations gave a Ca:Mg ratio of 1:1.90.

The annual losses of Ca-Mg ran somewhat in parallel, with progressive decrease from the first to the fourth year, the greatest differences occurring between the first and second years. The total enhancements of Ca-Mg outgo were comparable, ranging from 30.5 to 34.0 per cent of the amounts added.

Surface-zone versus subsurface-zone incorporation. The comparisons between outgo of calcium, magnesium and Ca-Mg from the two zones of incorporation are given for annual periods and for the 4-year period in table 3.

The maximum excess of lower-zone outgo over that from a corresponding upper-zone incorporation for any year was the 521-pound increase from $\text{Ca}(\text{OH})_2$ during the first year. This is equivalent to 55 per cent of that for the 4-year period. When corrected for the 357-pound outgo from the control the first-year losses attributable to treatment were 186 pounds and 707 pounds for the upper and lower zones, respectively, which gives from the lower zone an excess equivalent to 14.6 per cent of the full addition. Disparity between losses from the two zones decreased progressively thereafter. The high-magnesian limes showed comparable excess of outgo from the deep incorporations over the surface-zone incorporations for the first two years, with decided diminutions in differences for the last two years. As measured by the actual amounts of lime added, the high-calcic, burnt dolomite, and oxide mixtures show excesses in losses amounting to 26.3, 13.6 and 10.6 per cent, respectively, for the 4-year-period.

The earlier leachings from the treatments in the upper zone were rich in neutral calcium salts, which they carried to the lower zone, where there was no protective effect of any added lime. Magnesium replacement, with increased outgo, was accordingly effected in the untreated lower zone.

The only exception to the greater release of either alkali-earth from the lower-zone incorporations is observed in the repressed outgo of magnesium

TABLE 3

Influence of zone of incorporation of 2000-pound CaO equivalents (3570 pounds CaCO_3) of hydrated lime, burnt dolomite, and CaO-MgO upon outgo of total calcium, magnesium, and calcium-magnesium from a loam soil during annual and 4-year periods—terms of CaCO_3 -equivalence per 2,000,000 pounds of moisture-free soil

TREATMENT	FIRST YEAR						SECOND YEAR						THIRD YEAR						FOURTH YEAR						TOTAL FOR 4-YEAR PERIOD						
	Surface zone incor-		Subsurface zone in-		Excess outgo from		Surface zone incor-		Subsurface zone in-		Excess outgo from		Surface zone incor-		Subsurface zone in-		Excess outgo from		Surface zone incor-		Subsurface zone in-		Excess outgo from		Surface zone incor-		Subsurface zone in-		Excess outgo from		
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	per cent		
Calcium as CaCO ₃ ⇌																															
Ca(OH) ₂	543	1,064	521	220	438	218	188	314	126	190	265	75	1,141	2,081	940	26.3															
CaO-MgO*.....	506	634	128	188	315	127	195	216	21	177	188	11	1,066	1,353	287	13.6†															
CaO-MgO†.....	472	558	86	201	292	91	175	206	31	164	180	16	1,012	1,236	224	10.6‡															
Magnesium as CaCO ₃ ⇌																															
Ca(OH) ₂	152	96	-56	80	78	-2	65	58	-7	67	69	2	364	301	-63															
CaO-MgO*.....	138	490	352	78	249	171	89	176	87	94	181	87	399	1,096	697	47.9§															
CaO-MgO†.....	130	506	376	83	257	174	74	170	96	91	155	64	378	1,088	710	48.8§															
Calcium-magnesium as CaCO ₃ ⇌																															
Ca(OH) ₂	695	1,160	465	300	516	216	253	372	119	257	334	77	1,505	2,382	877	24.5															
CaO-MgO*.....	644	1,124	480	266	564	298	284	392	109	271	369	78	1,465	2,449	984	27.5‡															
CaO-MgO†.....	602	1,064	462	284	549	265	249	376	127	255	335	80	1,390	2,324	934	26.1‡															

* Calcinced dolomite, 46.53 per cent CaO, 32.85 per cent MgO—Supplying 1184.8 pounds CaO and 815.2 pounds CaO \rightleftharpoons , respectively.

† Mixtures of CaO and MgO from separately calcined marble and magnesite.

‡ Based on CaO content of high magnesian lime.

§ Based on MgO content of calcined CaO and calcined MgO mixture.

from the high-calcic lime incorporations. This may be readily accounted for by "reciprocal repression" (7). The leachings from the untreated zone lying above the lower-zone $\text{Ca}(\text{OH})_2$ incorporations should be comparable to those from the same zone in the tanks which received no additions to either zone. Hence, a repressive, or protective, effect upon the native magnesium of the lower zone was exerted by the deep incorporations of the high-calcic lime, the leachings from which passed directly from the tanks. This is in complete harmony with previous findings (5; 7, p. 450, 451) upon the same soil type, wherein it was shown that an absorbed excess of one alkali-earth may force back, or even inhibit, the tendency of its soluble neutral salts to effect interchange of the other alkali-earth.

The total excess of subsurface-zone magnesium outgo over that from the surface-zone incorporations was practically the same for the two magnesian limes. The magnesium excesses from the lower-zone additions of magnesium lime showed the same order—maximum for the first year and subsequent decreases—as that obtained in the calcium outgo from the high-calcic lime. It was shown (table 3) that the leachings from the surface-zone incorporations of CaO-MgO carried more calcium than magnesium, and it was stated that this was due to either lesser outgo of magnesium from the upper zone or greater magnesium absorption from the upper-zone leachings during their passage through the lower zone. In view of the greater outgo of magnesium from the same CaO-MgO incorporations in the lower zone, the latter explanation would seem to be more tenable.

The Ca-Mg totals from the three limes did not differ greatly in annual and 4-year intrazone comparisons. There was a consistent decrease in the disparity between greater outgo from the lower-zone and lesser outgo from the upper-zone incorporations after the first year, and in proportions greater than those to be anticipated from progressive decrease of residuals. The ratio of the group-average disparity in outgo for the first year to that for the second year was 2.57:1, whereas that for the second to third was 2.25:1, and that for the third to fourth 1.51:1, as accounting for the average increase in total outgo, which amounted to 26 per cent of the Ca-Mg added.

Nitrate and sulfate outgo

Surface-zone incorporations. The nitrate and sulfate leachings from surface-zone incorporations are given in table 4, as pounds of nitrogen and sulfur per 2,000,000 pounds of soil. At the beginning of the experiment the soil contained 0.1050 per cent, or 2100 pounds of nitrogen and 0.057 per cent, or 1140 pounds of sulfur on the same basis. The average annual increment of sulfur over a period of 9 years prior to the present experiment was 51.5 pounds (8), whereas that calculated from concomitant rain water was 180.4 pounds, or 45.1 pounds per annum.

The acceleration of nitrate outgo from the surface-zone incorporations of each of the three limes is attributable mainly to the increases during the first

TABLE 4

Nitrate nitrogen and sulfate sulfur leached during 4 years from 2000-pound CaO equivalences (3570 pounds CaCO₃) of hydrated lime, burnt dolomite and mixtures of separately calcined CaO and MgO incorporated in the surface zone of a loam soil—basis of 2,000,000 pounds moisture free soil

TREATMENTS	FIRST YEAR					SECOND YEAR					THIRD YEAR					FOURTH YEAR			TOTAL FOR 4-YEAR PERIOD	lbs.	4-YEAR INCREASE OVER CONTROLS
	May to September	September to January	January to March	March to May	Total	May to December	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total					
Nitrate nitrogen																					
Controls.....	20.8	32.1	3.3	6.9	63.1	32.4	2.3	2.4	37.1	21.1	11.2	1.8	34.1	29.6	5.1	34.7	169.0			
Ca(OH) ₂	28.9	47.6	4.9	6.7	88.1	41.7	3.5	1.6	46.8	20.9	4.1	3.3	28.3	29.6	2.3	31.9	195.1	26.1			
CaO-MgO*	34.8	37.6	3.9	2.5	78.1	37.9	1.8	1.0	40.7	25.2	9.9	3.2	38.3	26.9	2.7	29.6	186.7	17.7			
CaO-MgO†	39.1	45.7	4.4	3.9	93.1	39.2	2.8	1.4	43.4	20.0	5.7	3.2	28.9	26.5	2.4	28.9	194.3	25.3			
Sulfate sulfur																					
Controls.....	9.5	16.7	17.6	8.1	51.9	11.4	11.8	9.3	32.5	4.8	12.0	8.5	25.3	9.4	16.0	25.4	135.1			
Ca(OH) ₂	15.4	29.2	14.7	7.9	67.2	13.8	9.3	8.2	31.3	4.1	16.9	6.1	27.1	14.6	15.6	30.2	155.8	20.7			
CaO-MgO*	11.8	29.0	16.3	9.6	66.7	12.7	9.1	8.5	30.3	4.7	16.5	5.5	26.7	12.9	16.3	29.2	152.9	17.8			
CaO-MgO†	5.4	29.6	16.3	9.5	60.8	13.2	9.8	9.3	32.3	4.7	15.9	6.0	26.6	15.1	15.5	30.6	150.3	15.2			

* Calcined dolomite 46.53 per cent CaO 32.85 per cent MgO—Supplying 1184.8 pounds CaO and 815.2 pounds CaO $\frac{1}{2}$, respectively.

† Mixtures of CaO and MgO from separately calcined marble and magnesite.

TABLE 5

Nitrate nitrogen and sulfate sulfur leached during 4 years from 2000-pound CaO-equivalences (3570 pounds CaCO₃) of hydrated lime, burnt dolomite, and mixtures of separately calcined CaO and MgO incorporated in the subsurface zone of a loam soil—basis of 2,000,000 pounds moisture-free soil

TREATMENTS	FIRST YEAR				SECOND YEAR				THIRD YEAR				FOURTH YEAR				TOTAL FOR 4-YEAR PERIOD	4-YEAR INCREASE OVER CONTROLS			
	September to January		March to May		May to December		December to February		February to May		May to September		September to February		February to May				May to December		December to May
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Nitrate nitrogen																					
Controls.....	20.8	32.1	3.3	6.9	63.1	32.4	2.3	2.4	37.1	21.1	11.2	1.8	34.1	29.6	5.1	34.7	169.0
Ca(OH) ₂	38.9	35.7	3.6	6.4	84.6	26.7	1.4	0.8	28.9	24.9	3.1	3.2	31.2	26.6	2.3	28.9	173.6	4.6	4.6	4.6	4.6
CaO-MgO*	35.0	42.0	3.8	3.3	84.1	34.9	1.4	0.8	37.1	24.9	5.2	3.4	33.5	29.9	2.4	32.3	187.0	18.0	18.0	18.0	18.0
CaO-MgO†.....	31.9	44.7	4.0	4.7	85.3	33.2	0.7	0.6	34.6	18.7	4.9	2.6	26.2	27.3	4.0	31.3	177.4	8.4	8.4	8.4	8.4
Sulfate sulfur																					
Controls.....	9.5	16.7	17.6	8.1	51.9	11.4	11.8	9.3	32.5	4.8	12.0	8.5	25.3	9.4	16.0	25.4	135.1
Ca(OH) ₂	28.9	22.8	9.6	5.9	67.2	13.2	7.8	6.1	27.1	5.7	12.7	6.7	25.1	12.6	16.5	29.1	148.5	13.4	13.4	13.4	13.4
CaO-MgO*	29.1	24.5	10.7	5.4	69.7	14.0	7.6	7.7	29.3	6.6	12.6	6.6	25.8	12.7	14.9	27.6	152.4	17.3	17.3	17.3	17.3
CaO-MgO†.....	21.7	25.0	10.5	6.3	63.5	14.1	8.0	7.9	30.0	5.7	13.6	6.4	25.7	11.7	16.5	28.2	147.4	12.3	12.3	12.3	12.3

* Calcined dolomite 46.53 per cent CaO, 32.85 per cent MgO—Supplying 1184.8 pounds CaO and 815.2 pounds CaO ≈ respectively.

† Mixtures of CaO and MgO from separately calcined marble and magnesite.

year. After that period the incorporations gave nitrate losses comparable to, and even less than, the outgo from the control, especially during the third and fourth years. The variations between nitrate leaching from the three limes by years need not be stressed, since the maximum increase over the control amounts to only 26.1 pounds for the full 4-year period.

The records of sulfate outgo show the same initial acceleration shown in the case of nitrates during the first year. Only during this year did the sulfate leachings exceed the sulfate content of the rainfall. The concordance for the succeeding three years is very consistent, so that the maximum increase in sulfate leachings from each lime for the 4-year period is very close to that of the first year.

Subsurface-zone incorporations. The leachings from these incorporations are given in table 5. Comparable accelerations of nitrate outgo from subsurface-zone incorporations of the three limes were evidenced during the first year, but not thereafter; and when considered on the basis of average annual accelerations the maximum for any of the treatments was only 4.5 pounds. Acceleration in outgo as a result of mixing and aeration—and that due to this factor, plus treatment—was therefore most noticeable during the first year.

The largest sulfate outgo from the control and that from each of the three limes also came during the first year. The sulfate outgo from the controls equalled rainfall increment only during this year and only then did the three limes yield an amount in excess of that carried by rain waters. Thereafter the controls and the three caustic limes gave results so close that the respective 4-year totals were practically the same as their corresponding increases for the first year.

Surface-zone versus subsurface-zone incorporations

The variations between nitrate and sulfate losses from the two zones are given in table 6. Only in the case of the hydrated high-calcic lime was there an appreciable difference in the outgo of nitrate nitrogen from the two zones during the 4-year period. The plus and minus variations—with a maximum of 16.9 pounds, as a total for subsurface-zone over surface-zone incorporations—show that no extensive differences resulted from variation in depth of incorporation.

The sulfate differences were small, varying from plus to minus, and the differences between the totals for the 4-year period were insignificant. The average yield of total sulfur from the controls was, however, 45.4 pounds per acre less than that carried by rain water during the 4-year period. The average enhancement for all treatments in both zones was 16.1 pounds. Consequently, even with this enhancement in outgo, all sulfate leachings were uniformly less than the sulfate increment through rainfall.

From the foregoing it appears that the previously discussed differences in outgo of Ca-Mg were not to be accounted for by any material variation in

TABLE 6

Influence of zone of incorporation of 2000-pound CaO equivalents 3570) pounds CaCO₃ of hydrated lime, burnt dolomite, and CaO-MgO upon outgo of nitrate nitrogen and sulfate sulfur from a loam soil during annual and 4-year periods—basis of 2,000,000 pounds of moisture-free soil

TREATMENT	FIRST YEAR			SECOND YEAR			THIRD YEAR			FOURTH YEAR			TOTAL FOR 4-YEAR PERIOD		
	Surface zone incor-	Subsurface zone in-	Surface zone rela-	Surface zone incor-	Subsurface zone in-	Surface zone rela-	Surface zone incor-	Subsurface zone in-	Surface zone rela-	Surface zone incor-	Subsurface zone in-	Surface zone rela-	Surface zone incor-	Subsurface zone in-	Surface zone rela-
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Nitrate nitrogen															
Ca(OH) ₂	88.1	84.6	+3.5	46.8	28.9	+17.9	28.3	31.2	-2.9	31.9	28.9	+3.0	195.1	173.6	+21.5
CaO-MgO*.....	78.1	84.1	-6.0	40.7	37.1	+3.6	38.3	33.5	+4.8	29.6	32.3	-2.7	186.7	187.0	+0.3
CaO-MgO†.....	93.1	85.3	+7.8	43.4	34.6	+8.8	28.9	26.2	+2.7	28.9	31.3	-2.4	194.3	177.4	+16.9
Sulfate sulfur															
Ca(OH) ₂	67.2	67.2	Equal.	31.3	27.1	+4.2	27.1	25.1	+2.0	30.2	29.1	-1.1	155.8	148.5	+7.3
CaO-MgO*.....	66.7	69.7	-3.0	30.3	29.3	+1.0	26.7	25.8	+0.9	29.2	27.6	-1.6	152.9	152.4	+0.5
CaO-MgO†.....	60.8	63.5	-2.7	32.3	30.0	+2.3	26.6	25.7	+0.9	30.6	28.2	-2.4	150.3	147.4	+2.9

* Calcined dolomite 46.53 per cent CaO, 32.85 per cent MgO—Supplying 1184.8 pounds CaO and 815.2 pounds CaO ∞ , respectively.

† Mixtures of CaO and MgO from separately calcined marble and magnesite.

amounts of biological end-products. Rather, they were due primarily to greater hydrolysis of absorption complexes and greater leaching of the resultant bicarbonates of the two elements from the incorporations in the more moist lower zone.

Diminishing availability of absorbed calcium and Ca-Mg

The relationship between each total outgo and that for the first year and that of each succeeding year emphasized the point advanced (7, p. 461) in a related study with the same type of soil, where incorporations were made throughout the entire soil mass; namely, that the absorbed alkali-earths appear to resist hydrolysis more and more as they age. Earlier related studies showed that the calcium hydroxide additions must have been absorbed—directly from the hydroxide or after a part of the hydroxide had reverted to

TABLE 7

Influence of diminishment of residuals and of aging upon the availability of absorbed calcium from $\text{Ca}(\text{OH})_2$ and absorbed Ca-Mg from CaO-MgO incorporations at the constant rate of 2000 pounds of CaO (3570 pounds CaCO_3) per 1,000,000 pounds of subsurface-zone loam, as measured by Ca and Ca-Mg outgo, respectively—terms of pounds CaCO_3

LEACHING PERIOD	CALCIUM FROM $\text{Ca}(\text{OH})_2$ INCORPORATIONS				Ca-Mg FROM CaO-MgO INCORPORATIONS*			
	Present at beginning of period	Leached during period†	Outgo as related to residue of incorporation		Present at beginning of period	Leached during period†	Outgo as related to residue of incorporation	
			ratio	per cent			ratio	per cent
First year	3,570	707	1: 5.05	19.80	3,570	626	1: 5.70	17.53
Second year.	2,863	250	1:11.45	8.73	2,944	295	1: 9.98	10.02
Third year.	2,613	137	1:19.07	5.24	2,649	130	1:20.38	4.91
Fourth year.	2,476	89	1:27.82	3.59	2,519	102	1:24.70	4.05

* Average of burnt dolomite and separately calcined CaO and MgO.

† In excess of outgo from controls.

the carbonate—within a period of 10 days (1), and that at the close of the present experiment only small and near-equal amounts of carbonates remained (3, p. 410) from the $\text{Ca}(\text{OH})_2$ and CaO-MgO incorporations. It is evident that, if a study of influence of time upon availability is desired, it is essential that no untreated zone be interposed between the zone of treatment and the outlets for the leachings. This precludes the use of the upper-zone series in this connection. The series which received the $\text{Ca}(\text{OH})_2$ and CaO-MgO in the lower zone may, however, be utilized for this purpose.

Table 7 shows the excess of calcium lost from the lower-zone incorporation of $\text{Ca}(\text{OH})_2$ over the calcium outgo from the controls, and the average excess of Ca-Mg from the deep incorporations of the two types of magnesian lime. It also shows the fractions of the incorporations present at the beginning of each annual period, and the ratio and percentage relationships of such to each

annual enhancement in outgo. Table 2 shows that the amounts of calcium and magnesium lost from the controls were greatest during the first year, with near-constant outgo thereafter. It also shows that the maximum outgo of calcium from $\text{Ca}(\text{OH})_2$ and Ca-Mg from the CaO-MgO incorporations was greatest during the first year, with a marked decrease during the second year and further decreases during the succeeding two years. The maximum outgo of nitrates and sulfates during the first year fails to account for the loss of calcium and magnesium, and it follows that acceleration of CO_2 -production occurred in parallel with the production of the nitrates and sulfates. This has been established also by determinations of the bicarbonate content of the leachings. If this parallel of accelerated nitrate, sulfate, and CO_2 production had continued during the last three years, sufficient H_2CO_3 would have been available to maintain a bicarbonate outgo with a uniformity akin to that shown by the nitrate and sulfate leachings of table 5. But no such parallel in maintenance of outgo obtained. The losses of calcium from the $\text{Ca}(\text{OH})_2$ incorporations and the losses of Ca-Mg from the CaO-MgO incorporations suffered a decided decrease from year to year.

The ratios of calcium outgo to calcium addition, or its residual fraction, increased progressively from 1:5.05 for the first year to 1:27.82 for the fourth year, with conversely decreasing percentage extremes of 19.80 and 3.59. Similar extreme ratio values of 1:5.70 and 1:24.70 and percentage converse extremes of 17.53 and 4.05 obtain in expressing enhancements in Ca-Mg outgo as related to added CaO-MgO. The amount of calcium added as $\text{Ca}(\text{OH})_2$ —all but a small amount of which was quickly absorbed in non-carbonate combination—was 1.44 times that fraction which remained at the beginning of the fourth year; but the amount of calcium leaching during the first year was 7.85 times that leached during the fourth year. Or, at the beginning of the fourth year, the residue from the $\text{Ca}(\text{OH})_2$ incorporation was 69.4 per cent, whereas the enhancement in calcium leaching during the fourth year was only 12.6 per cent of that during the first year. The enhancement in outgo of Ca-Mg from the CaO-MgO incorporations shows the same general relationship between additions and annual residuals as those pointed out for calcium outgo and additions.

If the organic matter washed down by rain and that possibly derived from algae be disregarded, the only organic matter available for the generation of bacterial end-products during the experiment was the well-humified material present when the soil was placed. If the disintegration of this organic matter was at its maximum during the first year, and if thereafter it continued at the rate indicated by nitrate and sulfate outgo, diminishing residuals and their decreasing solubility would be the dominant causal factors in the progressive decrease in alkali-earth outgo during the last three years. But, if the amounts of CO_2 available to effect bicarbonate solutions of the hydrolyzed Ca-Mg decreased steadily, then the outgo of the two elements would have decreased, irrespective of the other two factors.

Expressed on the basis of full depth of surface soil, the initial CaCO_3 -equivalent of the non-carbonate calcium content of 9340 pounds was supplemented by 3570 pounds from the lower-zone incorporation of $\text{Ca}(\text{OH})_2$ at the beginning of the present experiment. The total uncorrected outgo from this augmented supply of 12910 pounds during the first year was 1064 pounds, or 8.24 per cent. Deducting from the initial supply of 12910 pounds the sum of the first year's outgo and the decreasing losses of 438 pounds and 314 pounds for the second and third years—i.e., 1816 pounds—there remained 11094 pounds at the beginning of the fourth year. If the same relation between total calcium content and outgo which obtained for the first year had continued throughout the first three years, the amount of calcium-carbonate-equivalent leached during the fourth year would have been 824 pounds, but the amount which did leach during the fourth year was only 265 pounds, or 2.66 per cent, of that present at the beginning of the fourth year. By using the surface-zone incorporation of $\text{Ca}(\text{OH})_2$ for the same method of calculation, the outgo for the first year was found to be 4.21 per cent of that present, whereas that for the fourth year was only 1.59 per cent. The outgo from the controls for the four years were, in order, 3.82, 2.09, 2.01, and 2.04 per cent of the amount present at the beginning of each of the respective years. Corresponding values of 8.24, 3.70, 2.75, and 2.39 per cent express the proportions of the four annual losses of calcium from that originally present plus the unleached fraction of the subsurface-zone incorporation of $\text{Ca}(\text{OH})_2$ present at the beginning of the respective years. If, however, the initial year of maximum outgo of all components be disregarded, and the 3.70 per cent of calcium outgo for the second year be applied to the residuals present at the beginning of the third and fourth years, on the assumption of uniform proportions between outgo and residues, the calculated values of 422 pounds and 410 pounds for the third and fourth years, respectively, are obtained. But the corresponding amounts actually leached were only 314 pounds and 265 pounds. These findings, together with the decrease of about one-half in outgo of calcium, magnesium, and Ca-Mg during the second year, and the approximation of the nitrate and sulfate outgo to that of the controls during the second, third, and fourth years, for both zones in all cases, indicate that enhanced outgo was accounted for in the main by biological accelerations only during the first year. In the absence of marked bacterial activities after that period, it appears that decreasing solubility—i.e., decreased hydrolysis—of the thoroughly disseminated absorption complexes was responsible for steadily decreasing outgo of alkali-earths. The combined Ca-Mg from the CaO-MgO incorporations develop corresponding relationships.

It is generally held that a second liming is required to maintain any benefits obtained from the initial application of the usual 2000-pound CaO-equivalent treatment, and it is usually thought that such reliming should be made after a period of from four to six years. It has been assumed that the need of

repetition was due to the loss of added lime through leaching and to its assimilation by plants. Such an assumption predicates that the absorption complexes derived from added lime are decidedly more soluble than those native to the soil. It is equally permissible to assume that the absorbed lime acquires in time the properties possessed by the native non-carbonate inorganic calcium compounds of the soil. From the foregoing it appears reasonable to assume that under the dynamic conditions prevalent in the soil system there is a tendency for the absorbed alkali-earths to become more and more insoluble, possibly more and more complex in make-up, and approaching in character the aged non-carbonate materials native to the soil. Under humid conditions this would obtain in greatest degree with a soil of the "heavier" type, containing materials sufficiently fine and acid to be designated as "acidoids" (10), the neutralization of which results in the fixation of added alkali-earths. With a soil having a minimum of such finely divided material, the fixation would not be extensive, greater leaching would occur, and additional lime would be needed to care for the paucity of alkali-earth nutrients. With a loam of the type used in this experiment, reliming in practice would probably be required to insure optimum reaction for biological activation and ready supplies of nutrients long before the removal of even a major part of the added lime; that is, most of the first addition of lime would still be present, but a second application would be beneficial. It appears most probable, therefore, that the function of such a supplemental supply of lime would be to furnish a "fresh stream" of alkali-earth of greater availability than that which had been previously absorbed, in order to offset progressive decrease in solubility, rather than the absolute supplementing of the total supplies of earthy materials and the replenishment of losses caused by leaching.

SUMMARY

Results are given from a 4-year lysimeter study of outgo of calcium, magnesium, Ca-Mg, nitrates, and sulfates from surface-zone and subsurface-zone incorporations of $\text{Ca}(\text{OH})_2$, burnt dolomite, and a corresponding CaO-MgO mixture, at a constant rate of 3570 pounds of CaCO_3 (2000 pounds of CaO) per 2,000,000 pounds of loam soil, moisture-free basis, as a result of an average annual rainfall of 51 inches.

The outgo of calcium from each lime was greatest during the first year, with progressive decrease thereafter, for both zones of incorporation. Because of a decidedly greater outgo from the subsurface-zone incorporation of $\text{Ca}(\text{OH})_2$, the conservation of lime exerted by the full soil depth upon the upper-zone incorporations was 940 pounds, or 26.3 per cent of the addition.

The magnesium outgo from the surface-zone incorporations of CaO-MgO was augmented during the first year, although practically the same as that from the controls during the succeeding three years; whereas it was enhanced

in all four annual leachings from the subsurface-zone incorporations, the maximum having been obtained during the first year.

The 4-year aggregates of Ca-Mg from the surface-zone incorporations of the three limes were comparable for each year and for the 4-year period. The totals of Ca-Mg from the subsurface-zone incorporations of the three limes were also comparable by yearly comparisons, with maximum for the first year and decreases thereafter. Each annual outgo, and that for the full period, was about 5 times as great as the corresponding one from incorporations in the upper zone.

Slight interchange of calcium for magnesium was found when the excess of calcium salts from the treated surface zone passed to the untreated lower zone, whereas "reciprocal repression" was noted in the direct leachings from the excess of absorbed calcium in the lower zone.

In general, accelerations in nitrate and sulfate outgo from CaO and CaO-MgO were appreciable and comparable during the first year. However, there was no marked variation thereafter, so that the respective increases for the 4-year period were uniformly about the same as the corresponding ones for the first annual period. Except during the first year, the nitrate and leachings were small during the winter months in all cases. No marked differences obtained as a result of difference in the zone of incorporation. Even with acceleration in sulfate outgo, the amount leached was, in no case, equivalent to the increment from rain water for the 4-year period.

The Ca-Mg proportion in the leachings from CaO-MgO varied with the zone of incorporation. Upper-zone leachings carried about twice as much calcium as magnesium, whereas the lower-zone incorporations yielded about twice as much magnesium as calcium. (These relationships correspond to those which obtained when finely ground dolomite was incorporated in the two zones of the same soil.)

With this particular soil and rate of incorporation, the principal differences resulting from equivalent incorporations of $\text{Ca}(\text{OH})_2$ and CaO-MgO were (a) variations in the proportion of calcium to magnesium in the leachings; (b) more extensive leachings of calcium and more extensive fixation of magnesium by the lower zone from equivalent quantities of calcium and magnesium; (c) Ca:Mg proportions of 1.7 to 1 in outgo from the upper-zone incorporation and 3 to 1 from the incorporations in the lower zone; and (d) somewhat greater calcium and Ca-Mg outgo from the burnt dolomite than from the corresponding separately calcined CaO-MgO, for both zones.

The progressive decrease in outgo of calcium and Ca-Mg is apparently not due so much to diminished residuals as to the diminishing solubility of such residuals, so that repetition of liming may become necessary, not primarily to offset outgo, but rather because of the need of more newly absorbed and more soluble non-carbonate forms, or undisintegrated carbonate residuals.

REFERENCES

- (1) MACINTIRE, W. H. 1919 The carbonation of burnt lime in soils. *Soil Sci.* 7: 351-356.
- (2) MACINTIRE, W. H. 1926 Influence of form, soil-zone and fineness of lime and magnesia incorporations upon outgo of: I. Calcium and magnesium. *Soil Sci.* 21: 377-391.
- (3) MACINTIRE, W. H., AND SHAW, W. M. 1925 The disintegration of limestone and dolomite separates as influenced by zone of incorporation. *Soil Sci.* 20: 404, 417.
- (4) MACINTIRE, W. H., AND SHAW, W. M. 1926 Fixation of calcium-magnesium from burnt limes, limestone and dolomite incorporations in two soil zones. *Soil Sci.* 22: 109-121.
- (5) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1923 Influence of calcic and magnesian treatments upon sulfate leachings from soil alone and with additions of ferrous sulfate, pyrite and sulfur. *Soil Sci.* 16: 172.
- (6) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1923 The variant rôles of soil and subsoil in calcium-magnesium interchange. *Soil Sci.* 16: 321-341.
- (7) MACINTIRE, W. H., SHAW, W. M., AND YOUNG, J. B. 1923 Reciprocal repression exerted by calcic and magnesian additions upon the solubility of native materials in surface soil. *Soil Sci.* 16: 449-464.
- (8) MACINTIRE, W. H., AND YOUNG, J. B. 1923 Sulfur, calcium, magnesium and potassium content and reaction of rainfall at different points in Tennessee. *Soil Sci.* 15: 207.
- (9) MACINTIRE, W. H., AND YOUNG, J. B. 1923 The transient nature of magnesium-induced toxicity and its bearing upon lime-magnesia ratio studies. *Soil Sci.* 15: 452.
- (10) MICHAELIS, L. 1925 The effects of ions in colloidal systems, p. 35, Williams and Wilkins, Baltimore.

POTASSIUM CONTENT OF PLANTS AS AN INDICATOR OF AVAILABLE SUPPLY IN SOIL.

J. W. AMES AND R. W. GERDEL

Ohio Agricultural Experiment Station

Received for publication May 28, 1926

Numerous investigations on the ash constituents of plants have been made from time to time in order to obtain information concerning available soil nutrients. Although the ash of certain plants has been found to vary with the soil in which they were grown, there are so many factors involved that the method may not furnish more reliable results than can be secured from a chemical analysis of the soil.

One of the more recent procedures proposed for using the plant as an analytical agent is the method described by Neubauer and Schneider (2), in which a large number of plants are grown for a limited time in a small amount of soil. The authors state that the method is based on the fact that seedlings do not live as long as possible upon the reserve materials of the seeds, but obtain nutritive substances from the soil through their rootlets as soon as they are developed.

It is considered that if a large number of young seedlings are grown in a small quantity of soil, the starving rootlets extract all the assimilable nutritive substances their strength permits, and thus separate the available from the non-available plant nutrients.

The details of the seedling method devised by Neubauer for ascertaining the fertilizer requirements of soils are essentially as follows:

In the bottom of a small glass dish having a diameter of approximately 11 cm. and a depth of 7 cm., is placed 100 gm. of carefully prepared soil, mixed with 50 gm. of pure sand. Over this soil and sand mixture the greater portion of 250 gm. of sand is spread, a sufficient amount for a $\frac{1}{2}$ -cm. covering over the seeds being reserved. A small glass tube for aeration and watering is placed in the center of the dish. After the dishes are prepared 100 carefully selected rye seeds are planted, water is added and the weight of the dishes obtained.

Neubauer states that the seed should weigh about 40 gm. to the one thousand, and be treated with a chlorphenol-mercury compound, "Upsulun." A kilogram of seed is treated for about $1\frac{1}{2}$ hours with a liter of a 0.1 per cent Upsulun solution containing 0.3 gm. sodium hydroxide, then dried on blotting paper and allowed to remain exposed to the air for a week. The seeds are germinated by keeping the dish in a moist warm place. After 17 days the seedlings with their rootlets are removed from the soil and sand, and the phosphorus and potassium contents determined.

Considerable attention has been given to the method in various publications, with special reference to its value for determining the fertilizer requirements

of sugar beets. Roemer (3) subjected a large number of soil samples to the Neubauer test in order to determine the ability of soils to satisfy the potassium and phosphate requirement of cultivated crops. The preliminary results of his investigation appear to be in accord with the claims of Neubauer.

In order to ascertain whether this method, or some modification of it embodying the essential features of nutrients utilized by plants, has possibilities for furnishing indications of supply or deficiency of available potassium in soils, experiments closely allied to this phase of the field of plant nutrition were undertaken.

EXPERIMENTAL

The soils used in this work were chiefly from several fertility experiment plots that have been fertilized and under observation for a period of years. Some of these soils had previously been studied to determine the effect of treatment and cropping on the solubility of potassium.

Several series of tests were made at different times, the proportions of soil and sand, the number of plants, and other conditions being varied somewhat.

In the first series rye was planted and the method of procedure followed was essentially the same as outlined by Neubauer, except that treatment of rye seed with chlorphenol-mercury solution was omitted. This resulted in unsatisfactory germination of the rye. Consequently the number of plants on different soils varied to such an extent that it was not considered worth while to analyze the plants from more than a few of the pots that produced a rather uniform number. Three portions of some of the 15 soils were used, and five or more of others, making a total of 57 pots.

The data for tops and roots weighed and analyzed separately, show that the ratio of tops to roots and the potassium removed by each vary appreciably. On account of the lesser weight and the greater bulk of the muck soil, 50-gm. portions of muck were mixed with sand and planted with 50 seeds. A calculation of the potassium removed from the muck soil on the same basis as from the mineral soils, indicates that it has no less available potassium. The data for this first series are given in table 1. The few results obtained from rye plants in this first experimental work with the method are of no special significance except that they emphasize the necessity of seed treatment to insure a uniform stand of plants.

Comparison of wheat and rye

On account of the unsatisfactory growth of rye in the first test, a comparison of wheat and rye was made by growing plants for 17 days in the same amounts of soil and sand, as recommended by Neubauer except that 80 seeds instead of 100 were planted. The seeds were treated with chlorphenol-mercury compound before planting.

The data in table 2 show that wheat produced larger weights of plants containing increased amounts of potassium. The weights of potassium in

roots of wheat plants in the two soils were also larger, but there was no consistent variation in roots of wheat and rye grown in pure sand. The greater

TABLE 1
Rye plants of first series; grown for 14 days

SOIL	NUMBER OF PLANTS	WEIGHTS OF DRY		POTASSIUM REMOVED BY		POTASSIUM CONCENTRATION	
		Tops	Roots	Tops	Roots	Tops	Roots
		gm.	gm.	mgm.	mgm.	per cent	per cent
Forestry plot.	60	0 609	1 089	12.3	6 3	2 00	0.59
	62	0.520	1 212	11 4	6 2	2.16	0.52
	62	0.529	1 222	12 2	6.9	2 31	0.57
Paulding.	96	0 837	1 434	16 1	8.0	1 92	0.56
	95	0 839	1 089	14.1	10.4	1.69	0.96
Muck.	84	0 790	0 942	9 8	6 4	1 24	0.69
	94	0 840	1 080	11.2	6.4	1.33	0.59
	80	0.822	0.978	11.2	4.6	1 38	0.48
Sand.	91	0.652	1.180	11 0	7.4	1.54	0.63
	88	0.598	1.114	10 2	6 7	1.71	0.59
	91	0.606	1.138	7 7	7 3	1 27	0.64

TABLE 2
Comparison of wheat and rye

SOIL	CROP	NUMBER OF PLANTS	WEIGHT OF TOPS	WEIGHT OF ROOTS	POTASSIUM IN		POTASSIUM CONCENTRATION	
					Tops	Roots	Tops	Roots
			gm.	gm.	mgm.	mgm.	per cent	per cent
Pure sand.	Rye	79	0 5871	1 1452	5 2	5 1	0.889	0.443
	Rye	74	0.5500	0 7955	4 5	4.2	0.818	0.522
	Wheat	80	0 8177	1.2716	7 5	5 7	0.915	0.349
	Wheat	80	0.8057	1.2542	6.6	5.1	0 818	0.404
Silt loam.	Rye	70	0.5477	0 6018	7 2	3 0	1 321	0 506
	Rye	73	0.6027	0.7077	7.1	3.7	1.173	0.518
	Wheat	80	0.8373	1.1388	9.7	6 2	1.162	0.545
	Wheat	74	0.7745	1 1247	10.7	6.6	1.380	0.589
Clyde sand.	Rye	72	0.7276	0.9045	21.0	5.4	2.884	0.595
	Rye	80	0.7268	0.8694	24.0	7.0	3.363	0 809
	Wheat	73	1.0000	1.3127	24 0	9.2	2 429	0.920
	Wheat	80	1.0300	1.4364	27.0	9.0	2.623	0.875

weights of plants grown in the Clyde sandy soil indicate that conditions for growth were more favorable than in the silt loam soil taken from a nitrogen-

and phosphorus-fertilized plot of a 3-year rotation fertility experiment field on the Wooster farm. The potassium content of plants grown in Clyde sand was appreciably greater than that of plants grown in silt loam soil, although

TABLE 3
Potassium removed from several soils by wheat plants

SOIL AND TREATMENT	DRY WEIGHT		POTASSIUM IN			POTASSIUM CONCENTRATION		
	Tops	Roots	Tops	Roots	Entire plant	Tops	Roots	Entire plant
	gm.	gm.	mgm.	mgm.	mgm.	per cent	per cent	per cent
Wooster silt loam; * no treatment . . .	0 934	0 934	10 4	4 8	15.2	1.112	0.51	0.814
Wooster silt loam, limed	0 823	0.882	9 4	5 1	14 5	1.141	0 58	0 851
Wooster silt loam; acid phosphate fertilizer.....	0 919	0 885	10.7	4 7	15 4	1.163	0.53	0 855
Wooster silt loam; acid phosphate fertilizer; limed.	0 910	0 988	9.4	6 0	15 4	1 030	0.59	0.809
Wooster silt loam; muriate potash fertilizer.....	0 999	0 817	15 0	5.4	20.4	1.520	0 66	1 130
Forestry soil	1 040	0 859	29 7	6 0	35 7	2.840	0 73	1 882
Clyde clay	0 962	0 869	18 8	7.0	25.8	1 941	0 82	1 383
Clyde sand	0 833	0.865	12 6	4 2	16 8	1.510	0 49	0.992
Truck farm soil	0 910	0 902	13.9	5.0	18 9	1.481	0 61	1.044
Muck	0.811	0 853	11 0	3 9	14 9	1.330	0.49	0 877
Pure sand.	0.815	0 949	7 9	4 2	12 1	0.980	0.44	0 687

* Treatment on Wooster silt loam has been as follows:

Lime has been applied in varying amounts since 1903.

Acid phosphate 320 pounds each 5-year period since 1894.

Muriate potash 260 pounds each 5-year period since 1894.

TABLE 4
Soil potassium soluble in 0.01 N HNO₃

SOIL AND TREATMENT	SOLUBLE POTASSIUM
	p.p.m.
Wooster silt loam; no treatment.....	34
Wooster silt loam; limed.	23
Wooster silt loam; acid phosphate fertilizer.....	37
Wooster silt loam; acid phosphate fertilizer; limed	17
Wooster silt loam; muriate potash fertilizer.....	77
Forestry soil	88
Clyde clay	36
Truck farm soil	63

sandy soils are considered to be more responsive to potassium additions. These results with the two grains indicated that wheat was more satisfactory than rye.

Test of wheat grown in 100 gm. of soil

Table 3 gives results obtained by applying the Neubauer test to several soils. In this work 100 gm. of soil mixed with 50 gm. of sand was placed in the bottom of small pots and covered with 250 gm. of sand. Information regarding the dilute acid-soluble potassium content of some of the soils included in this test had previously been obtained from 0.01 normal acid extractions. The acid-soluble potassium figures are given in table 4. Wheat plants withdrew the largest amount from the Forestry soil, which contained the most soluble potassium. This soil is a part of the Wooster farm that has not been cropped, but that had been used as a forestry nursery for a few years and that has been treated with manure. The Clyde clay, with a high content of total potassium, contained no more dilute acid-soluble potassium than the unfertilized silt loam on which certain crops make a more decided response to potash fertilizer. Potassium removed by wheat plants from the Clyde clay soil, however, gave indications that this soil has an abundance of available potassium. This agrees with the crop response to potash in field practice. Removal of potassium by wheat plants from limed and unlimed portions of Wooster silt loam soil fertilized with acid phosphate does not give the same indication of less available potassium in limed soil that was obtained from dilute acid extraction of the soil. Potassium taken up by wheat seedlings from potash-fertilized plot reflects the larger amount of soluble residual potassium.

Varying proportions of soil and number of plants

Some wheat plants grown in pots of a larger diameter, 5 inches, and having a capacity for approximately 1000 gm. of sand made a more vigorous growth than plants in containers of sufficient size for 100 gm. of soil and 300 gm. of sand. The soil was not placed in the bottom of the pot as suggested by Neubauer, but was mixed with the total amount of sand. From this it was concluded that a larger volume, permitting more extensive root development, was an advantage, and a test was made to determine what soil dilution and number of plants would provide the most favorable condition for obtaining indications of available potassium. In this work 25, 50, and 100 wheat plants were grown in 1000 gm. of sand containing the amounts of soil stated in table 5. Potassium assimilated from the soil is represented by the amount in a given number of plants less that in the same number of plants grown in pure sand. These data for the actual amount of potassium assimilated from the soil are given in column 6 of table 5.

It will be noted that in no case is the excess of potassium directly proportional to the number of plants grown in a given soil dilution. Neither is there any correlation between potassium removed by a given number of plants and the degree of soil dilution. In fact the data indicate that 25 plants on 50 gm. of soil assimilate proportionately more of the soil potassium than do 50 plants

on 100 gm. of soil. For analytical purposes, 25 or 50 wheat plants do not produce sufficient material. The optimum number is 100. It was concluded

TABLE 5

Averaged results for wheat plants grown in varying proportions of soil mixed with 1000 gm. sand

SOIL	NUMBER OF PLANTS	DRY WEIGHT OF PLANTS	POTASSIUM IN PLANTS	POTASSIUM CONCENTRATION	POTASSIUM REMOVED IN EXCESS OF THAT IN SEEDS
gm.		gm.	mgm.	per cent	mgm.
50	25	0.823	5.8	0.699	2.1
50	50	1.547	10.0	0.649	2.0
50	100	2.801	13.8	0.492	0.2
100	25	0.881	5.6	0.632	1.9
100	50	1.579	8.9	0.566	0.9
100	100	2.754	14.9	0.541	1.3
200	25	0.937	6.7	0.714	3.0
200	50	1.698	9.2	0.598	1.2
200	100	3.205	17.6	0.548	4.0
Sand*	25	0.743	3.7	0.500	
Sand	50	1.409	8.0	0.568	
Sand	100	2.656	13.6	0.513	

* Results for sand representing available in seed.

TABLE 6

Potassium removed from fertility plot soils by wheat plants

SOIL TREATMENT IN FIELD*	DRY WEIGHT OF TOPS	POTASSIUM IN TOPS	POTASSIUM CONCENTRATION
	gm.	mgm.	per cent
None.....	1.149	8.5	0.738
Lime	1.351	12.1	0.896
Acid phosphate.....	1.593	14.5	0.908
Acid phosphate; lime.....	1.253	12.4	0.959
Muriate potash	1.388	17.2	1.142
Muriate potash; lime	1.354	18.9	1.397
Nitrate soda.....	1.111	11.9	1.068
Nitrate soda; lime.....	1.116	11.6	1.038
Manure.....	1.293	15.5	1.199
Manure: lime.....	1.367	16.2	1.189

* Lime as been applied in varying amounts since 1903. Fertilizer and manure additions for each 5-year period since 1894 have been as follows: Acid phosphate, 320 pounds; muriate potash, 260 pounds; nitrate soda, 440 pounds; manure, 16 tons.

from this test of soil dilutions that 200 gm. of soil in 1000 gm. of sand is better than a smaller amount, since this dilution produced the greatest amount of

plant material associated with the largest removal of potassium. It was found that the potassium content of 3.5 gm.—the average weight of 10 sets of 100 seeds—was 16.33 mg. This is in excess of the potassium taken up by plants grown in pure sand, and in plants grown in 50 or 100 gm. of soil.

Test of fertility plot soils

Some further tests with the seedling method were made of soils from several of the 5-year rotation fertility plots (4). Wheat plants were grown in 200 gm. of soil diluted with 1000 gm. of sand. On account of the difficulty of removing adhering soil from the roots, only the tops were weighed and analyzed.

The data for plants grown under these conditions are given in table 6. Inspection of the results shows that plants removed more potassium from soils of plots that contain potassium residual from muriate of potash and manure. Aside from these two soils there is no very significant relation between potassium removal and the fertilizer treatment.

Influence of additions

Usually when vegetation tests are made for the purpose of determining variations in supply of some essential element, it is considered necessary to insure the optimum condition for the other growth factors, by supplying an excess of the nutritive substances that are not under consideration. Although Neubauer states that this does not appear to affect the abstraction of nutritive substances by seedlings, some tests were made to determine what effect additions to soil would have on potassium removal from fertility plot soils that have been variously treated for a long continued period. In one series, available nitrogen was supplied by applying 0.143 gm. of sodium nitrate or an equivalent amount of ammonium sulfate to 200 gm. of soil mixed with 1000 gm. of sand. This amount is equivalent to 120 p.p.m. of sand and soil mixture. These nitrogen-carrying salts in solution were added to the soil 10 days previous to incorporating it with sand and planting the wheat. Water was added to bring the moisture content to 12 per cent, which was maintained throughout the experiment by daily additions through a small glass tube placed in the center of each pot.

The tabulated data for this series given in table 7 are the averaged results for triplicate pots of each treatment. As the weights of plant material produced, undoubtedly have an influence on the amount of potassium removed and its concentration in the plant, attention is directed to the influence of soil and of treatment on variations in weight of 100 plants grown on the several soils. Ammonium sulfate, on practically all of the soils, adversely affected growth of plants, as the weights of dry matter are less than for plants from pots receiving no additions of nitrate of soda or ammonium sulfate. This effect of ammonium sulfate was generally most pronounced on soils from unlimed portions of plots. Whether this effect was caused by increased

TABLE 7

Effect on wheat plants of additions of sodium nitrate and ammonium sulfate to fertility plot soils

SOIL TREATMENT IN FIELD*	POT ADDITION	DRY WEIGHT OF TOPS	POTAS- SIUM IN TOPS	POTAS- SIUM CONCEN- TRATION
		gm.	mgm.	per cent
No treatment	Sodium nitrate	1 280	12.7	1.007
	Ammonium sulfate	0 988	10.7	1.084
	No addition	1.149	8.5	0.738
Lime	Sodium nitrate	1.403	13.8	0.982
	Ammonium sulfate	1.235	13.5	1.088
	No addition	1.351	12.1	0.896
Acid phosphate	Sodium nitrate	1.532	15.6	1.016
	Ammonium sulfate	1.282	15.3	1.191
	No addition	1.593	14.5	0.908
Acid phosphate; lime	Sodium nitrate	1.381	13.9	0.989
	Ammonium sulfate	1.206	13.1	1.081
	No addition	1.253	12.4	0.959
Muriate potash	Sodium nitrate	1.726	20.3	1.179
	Ammonium sulfate	1 162	21.0	1.809
	No addition	1.388	17.3	1.142
Muriate potash; lime	Sodium nitrate	1.218	14.5	1.211
	Ammonium sulfate	1.159	14.5	1.254
	No addition	1.354	18.9	1.397
Nitrate soda	Sodium nitrate	1 163	12.8	1.104
	Ammonium sulfate	0.976	12.7	1.301
	No addition	1.111	11.9	1.068
Nitrate soda; lime	Sodium nitrate	1.312	11.0	0.840
	Ammonium sulfate	1.098	11.4	1.033
	No addition	1.116	11.6	1.038
Manure	Sodium nitrate	1.359	18.2	1.341
	Ammonium sulfate	1.049	16.6	1.584
	No addition	1.293	15.5	1.199
Manure; lime	Sodium nitrate	1.425	17.5	1.224
	Ammonium sulfate	1.385	17.4	1.262
	No addition	1.367	16.2	1.189

* Fertilizer and manure treatment of plots as stated in footnote table 6.

acidity or by physiological unbalance is not evident. Nitrate of soda produced more vigorous growth and a larger weight of plant material on all soils except those from the plot treated with acid phosphate and from the limed portion of the plot fertilized with muriate of potash. The comparative effect of sodium nitrate and ammonium sulfate on growth of wheat plants in this experiment is shown in plate 1.

It will be noted that although the yield of potassium in plant material was in some cases associated with a larger weight of plants, this was not uniformly so. Both sodium nitrate and ammonium sulfate additions to the limed and unlimed portions of unfertilized soil caused an increased potassium assimilation over that of plants from soil receiving no addition; this was true regardless of the dry weight, which was lowest with the ammonium sulfate treatment. In the case of unlimed soil from the acid phosphate fertilized plot, the pot with no addition gave the heaviest growth of plants, but the amount of potassium assimilated falls below that from the treated pots. Although ammonium sulfate has caused a decreased weight of plants on this soil, potassium removal was about the same as that of the plants grown in the pot that had an addition of sodium nitrate.

These additions also caused an increased potassium assimilation or removal from soils of the other plots, excepting from limed portions of plots fertilized with nitrate of soda and muriate of potash. The results for the latter plot show that the amount of potassium in plants from the pot that had no addition was noticeably more than in plants grown on this soil modified by additions of sodium nitrate and ammonium sulfate.

From the results for unlimed soil fertilized with potash it is quite apparent that this soil contains considerable residual potassium, and that both sodium nitrate and ammonium sulfate have increased the removal by wheat plants. Possibly base exchange may have been a factor influencing the larger removal of potassium from this soil. Ammonium sulfate retarded plant growth on this soil whereas nitrate of soda increased it, which accounts for the extreme variation in percentage or concentration of potassium in the plant material. When the growth medium was soil from the limed portion of the potash fertilized plot the results display an almost complete reversal of those obtained for the other soils of this series, for nitrate of soda and ammonium sulfate have not only retarded vegetative growth, but have also appreciably reduced the amount of potassium assimilated. An expansion of this may be that in the absence of other assimilable elements the plant draws more heavily upon an element available in larger quantity.

The potassium content of plants grown on manured soil furnishes indications of an available supply that is somewhat less than that in muriate of potash fertilized soil, but more than in the other soils.

Although the variations in potassium assimilation of wheat plants grown under modified conditions of treatment are not of sufficient magnitude to justify positive deductions, it would seem that the quantity of potassium in

wheat plants at the seedling stage is in a measure influenced by nutritional growth factors. If the results for plants from soils treated with ammonium sulfate are omitted, it is apparent that increased plant growth and content of potassium are closely in accord.

It is a question whether the total amount of potassium in plants or the concentration gives the more correct indications of available supply, or of the plant's capacity to assimilate potassium when growth factors are variable. Obviously the calculated concentration of potassium is dependent on the weight of plant material, so that although approximately equal amounts of potassium may be present in the same number of plants grown in the same quantity of soil, small differences in weights may affect the results calculated on a percentage basis. Neubauer apparently does not make deductions from concentration of potassium in plants.

A further test of the effect of available nutrients on plant growth and assimilation of potassium, included additions of mono-calcium phosphate and potassium chloride, as well as sodium nitrate and ammonium sulfate to several soils from fertility plots. In this series the proportions of soil and sand were the same as in the previous test, and the water content was maintained at 12 per cent. The amounts of salts added in solution to soils previous to diluting with sand and placing in pots were as follows: mono-calcium phosphate, 0.075 gm.; potassium chloride, 0.15 gm.; sodium nitrate, 0.143 gm.; ammonium sulfate, 0.108 gm.

Mono-calcium phosphate furnished phosphorus equivalent to that in 250 pounds of acid phosphate per million of soil on the basis of sand and soil mixture. Potassium chloride at the rate of 0.15 gm. to a pot is equal to 125 pounds per million of soil. Sodium nitrate, 0.143 gm. per pot, is at the rate of approximately 120 pounds to one million of soil; 0.108 gm. of ammonium sulfate is equal to about 90 pounds per million of soil.

The wheat seedlings were grown for 20 days. The data for this series in table 8 exhibit decided variations in weights of dry plant material and potassium removal from the several soils included in this test. Ammonium sulfate and mono-calcium phosphate in contrast with sodium nitrate or potassium chloride additions on the same soil, adversely affected plant growth, so that less weight of plant material was produced. Sodium nitrate was most effective in promoting plant growth on soil from the plot previously fertilized with muriate of potash. This was also true for the previous series where sodium nitrate was added to soil from this plot.

Potassium chloride had a marked influence on stimulating the growth of wheat seedlings. Compared with the plants of the previous series grown in pots that had no addition, the weights of plants that received potassium chloride were consistently increased. The remarkable stimulation of growth on unfertilized soil by potassium chloride in contrast with phosphate, ammonium sulfate, and sodium nitrate additions is illustrated in plate 2. The growth made by plants on unfertilized soil and on soil from plots that have

TABLE 8

Effect on wheat plants of addition of mono-calcium phosphate, potassium chloride, sodium nitrate, and ammonium sulfate to soils

SOIL AND TREATMENT IN FIELD*	POT ADDITIONS	DRY WEIGHT OF TOPS	POTASSIUM IN TOPS	POTASSIUM CONCENTRATION
		gm.	mgm	per cent
5-year rotation. No treatment	Sodium nitrate	1 218	7.5	0.614
	Ammonium sulfate	1 034	8.4	0.805
	Mono-calcium phosphate	1.234	11 1	0.910
	Potassium chloride	1.825	65.2	3.583
5-year rotation. Lime	Sodium nitrate	1.447	11.2	0.817
	Ammonium sulfate	1 175	11.2	0.979
	Mono-calcium phosphate	1.233	12.9	1.051
	Potassium chloride	1 773	65.0	3.666
5-year rotation. Acid phosphate	Sodium nitrate	1 549	14 7	0.951
	Ammonium sulfate	1.534	8.9	0.582
	Potassium chloride	2.038	73 3	3.596
5-year rotation. Acid phosphate; lime	Sodium nitrate	1 419	12.9	0.907
	Ammonium sulfate	1 371	7.9	0.575
	Potassium chloride	1 469	43.5	2.968
5-year rotation. Muriate potash	Sodium nitrate	1 891	22.6	1.195
	Ammonium sulfate	1 493	16 9	1.136
	Mono-calcium phosphate	1 341	21.2	1.581
5-year rotation. Muriate potash; lime	Sodium nitrate	1.575	19.3	1.226
	Ammonium sulfate	1.355	13 8	1.021
	Mono-calcium phosphate	1.212	19 1	1.581
5-year rotation. Nitrate soda	Sodium nitrate	1.534	14 2	0.928
	Ammonium sulfate	1.224	13.4	1.097
	Mono-calcium phosphate	1.250	16.4	1.315
	Potassium chloride	1.491	60.5	4.061
5-year rotation. Nitrate soda; lime	Sodium nitrate	1.601	14.6	0.914
	Ammonium sulfate	1 268	11.8	0.932
	Mono-calcium phosphate	1.348	14.3	1.057
	Potassium chloride	1.581	63.4	4.408
5-year rotation. Acid phosphate; nitrate soda	Mono-calcium phosphate	1.228	12.4	1.053
	Potassium chloride	1.525	35.1	2.301
5-year rotation. Acid phosphate; nitrate soda; lime	Mono-calcium phosphate	1.262	14.6	1.161
	Potassium chloride	1 757	70.0	4.009

* Fertilizer treatment of plots for each 5-year period since 1894 as follows: Acid phosphate 320 pounds per acre; muriate of potash 260 pounds; nitrate of soda 440 pounds; manure 16 tons. Treatment for each 3-year rotation period since 1896; Acid phosphate 320 pounds per acre; nitrate of soda 200 pounds.

TABLE 8—*Concluded*

SOIL AND TREATMENT IN FIELD*	POT ADDITIONS	DRY WEIGHT OF TOPS	POTASSIUM IN TOPS	POTASSIUM CONCENTRATION
		gm.	mgm.	per cent
5-year rotation. Manure	Mono-calcium phosphate	1.232	17.7	1.433
5-year rotation. Manure; lime	Mono-calcium phosphate	1.281	17.0	1.326
3-year rotation. No treatment	Potassium chloride	1.805	84.7	4.688
3-year rotation. Acid phosphate	Potassium chloride	1.569	74.7	4.763
3-year rotation. Acid phosphate; nitrate soda	Potassium chloride	1.558	81.9	5.274
Forestry plot soil	Potassium chloride	2.113	82.0	3.919

been fertilized singly with nitrate of soda, acid phosphate, and muriate of potash can be seen from the photographs of wheat plants (plate 3). On the muriate of potash fertilized soil that had no additions at the time of growing the wheat seedlings, the residual potassium evidently stimulated the growth of the young wheat plants. Although addition of sodium nitrate caused a greater weight of plants on several of the soils, the removal of potassium was not increased except from the soils of plots previously fertilized with muriate of potash and nitrate of soda.

Mono-calcium phosphate apparently increased the amount of potassium in wheat seedlings. Regardless of the effect of this addition on the growth and weight of plants, larger amounts of potassium, and increased concentration over untreated soil resulted where mono-calcium phosphate was added.

Potassium chloride had the most marked influence on potassium content, and also appreciably increased the weights of plants grown on several soils. Although the amount added was comparatively small—0.078 gm.—the young plants assimilated potassium in excess of their requirements. From certain of the soils, the plants contained potassium in excess of the soluble potassium added, which would seem to indicate 100 per cent recovery.

The data for plants grown on soil that had previously been fertilized with muriate of potash indicate a luxury consumption of potassium, since an increased potassium content is not always associated with greater weight of plants.

The results for some of the treatments show that there is an inverse relation between plant weight and potassium content. If the growth of plants is influenced by the supply or deficiency of one or more essential elements, the amount of potassium in plants may vary, although the amount is the same in different soils or in the same soil modified by treatment.

Potassium assimilated by wheat and buckwheat

In this test 10 plants to a pot were grown in different soil dilutions for 20 days. Plants were grown in 50, 100, and 200 gm. of soil mixed with sufficient sand to give a total weight of 1600 gm. in each pot. Plants were also grown in pure quartz sand and in soil with no dilution. The results obtained from wheat and buckwheat are given in table 9.

It will be noted that in 200 gm. of soil the increased weight of buckwheat plants was more marked, but with smaller amounts of soil the differences between weight of wheat and of buckwheat decreased.

With less than 200 gm. of soil the potassium content of 10 wheat plants was the same, but there were gradually increasing amounts of potassium in the buckwheat, resulting in a rather consistent relation between the production of plant material and the potassium assimilated. It is evident from the amounts of potassium removed by wheat and buckwheat grown for the same length of time, that buckwheat draws more heavily on soil potassium. The

TABLE 9

Data for wheat and buckwheat plants grown in varying proportions of soil and sand

WEIGHT OF SOIL	DRY PLANT WEIGHT		POTASSIUM IN TOPS		POTASSIUM CONCENTRATION	
	Wheat	Buckwheat	Wheat	Buckwheat	Wheat	Buckwheat
gm.	gm.	gm.	mgm.	mgm.	per cent	per cent
0	0.303	0.307	3.8	3.7	1.246	1.200
50	0.358	0.551	3.7	5.1	1.032	0.918
100	0.433	0.761	3.9	7.3	0.909	0.961
200	0.669	1.251	5.7	14.2	0.860	1.131
1,600	1.977	3.579	22.8	49.5	1.151	1.384

more marked increased growth of buckwheat as compared with wheat in the several soil dilutions is shown in plate 4. Some preliminary experiments reported by Haley (1) have indicated that buckwheat is satisfactory for measuring the availability of potassium in soils.

Potassium removal by corn plants

In some further work, corn plants were grown in soil from fertility experiment plots. In one test 5 corn plants were grown for 18 days in 800 gm. of soil diluted with 4000 gm. of sand. The soils were from unlimed and limed portions of an unfertilized plot, and from plots that had been fertilized with muriate of potash and with acid phosphate. From the results in table 10 it is apparent that sodium nitrate and ammonium sulfate added to supply available nitrogen and bases for replacement reactions apparently have had little or no effect.

The more marked variations in potassium removed were found in the corn plants grown on soil from plots fertilized with acid phosphate and with muriate

TABLE 10

Potassium removal by 5 Corn Plants grown for 18 days in 800 gm. soil mixed with 4000 gm. sand

SOIL TREATMENT ON PLOTS*	POT ADDITIONS	WEIGHT		POTASSIUM REMOVED	
		Tops	Roots	Tops	Roots
		gm.	gm.	mgm.	mgm.
None.....	None	1.11	2.05	13	12
	Sodium nitrate	1.18	2.12	12	11
	Ammonium sulfate	1.05	2.13	9	7
Lime.....	None	1.16	2.48	17	17
	Sodium nitrate	1.11	2.17	13	14
	Ammonium sulfate	1.31	2.57	23	16
Acid phosphate.....	None	0.92	1.60	8	8
	Sodium nitrate	0.87	1.84	9	6
	Ammonium sulfate	0.81	1.64	8	7
Acid phosphate; lime.....	None	1.06	1.84	8	9
	Sodium nitrate	0.82	1.79	7	8
	Ammonium sulfate	0.84	1.83	8	7
Muriate potash.....	None	0.93	1.72	21	15
	Sodium nitrate	1.02	1.67	22	17
	Ammonium sulfate	1.07	1.84	26	22
Muriate potash; lime.....	None	0.91	1.79	15	14
	Sodium nitrate	0.73	1.73	11	10
	Ammonium sulfate	0.72	1.56	10	9
Sand.....	None	0.29	1.27	4	5

* Treatment of plots same as stated in footnote table 6.

TABLE 11

Potassium removed by corn and wheat plants grown for 18 days

SOIL TREATMENT IN FIELD	POTASSIUM REMOVED BY		CENTI-NORMAL ACID-SOLUBLE POTASSIUM <i>p.p.m. soil</i>
	Wheat	Corn	
	mgm.	mgm.	
None.....	8	13	34
Lime.....	12	17	23
Acid phosphate.....	14	8	37
Acid phosphate; lime.....	12	8	17
Muriate potash.....	17	21	77
Muriate potash; lime.....	19	15	36

of potash. In table 11, the results for 5 corn plants grown in 800 gm. of soil diluted with 4000 gm. of sand are compared with the removal by 100 wheat plants grown in 200 gm. of soil and 1000 gm. of sand. Although the ratio of soil to sand was the same for corn and wheat the former had more soil,

TABLE 12

*Potassium removed by 2 corn plants grown in undiluted soil from fertility experiment plots—
Planted June 3, cut July 25*

SOIL AND FERTILIZER TREATMENT*	POT ADDITIONS	WEIGHT OF TOPS	POTAS- SIUM REMOVED	POTAS- SIUM CONCEN- TRATION
		gm.	mgm.	per cent
<i>5-year rotation:</i>				
None.....	None	15	154	1.025
None.....	Acid phosphate; sodium nitrate	25	188	0.752
None.....	Acid phosphate; sodium nitrate; potassium chloride	30	512	1.705
Acid phosphate.....	None	18	146	0.812
Acid phosphate.....	Potassium chloride	25	346	1.383
Acid phosphate.....	Sodium nitrate; potassium chloride	17	281	1.653
Muriate potash.....	None	15	311	2.075
Muriate potash.....	Acid phosphate	20	343	1.717
Muriate potash.....	Acid phosphate; sodium nitrate	20	332	1.661
Acid phosphate; sodium nitrate...	None	15	148	0.989
Acid phosphate; sodium nitrate...	Potassium chloride	19	289	1.520
<i>3-year potato rotation:</i>				
None.....	None	19	177	0.929
None.....	Potassium chloride	22	319	1.452
Acid phosphate.....	None	15	116	0.772
Acid phosphate.....	Potassium chloride	20	286	1.431
Acid phosphate.....	Potassium chloride; sodium nitrate	30	362	1.206
Muriate potash.....	None	15	259	1.725
Muriate potash.....	Acid phosphate; sodium nitrate	25	277	1.109
Forestry plot	None	32	678	2.119
Forestry plot.....	Acid phosphate	39	756	1.938

* Fertilizer treatment of plots for each 5-year period since 1894 as follows: Acid phosphate 320 pounds per acre; muriate potash 260 pounds; nitrate soda 440 pounds. Treatment for each 3-year rotation period since 1896: Acid phosphate 320 pounds; muriate potash 200 pounds.

and consequently more potassium at its disposal. If the dilute-acid-soluble potassium furnishes reliable evidence of variations in availability, the corn plant in this test has been no more satisfactory than wheat in furnishing indications of the characteristics of the soil in this respect.

Potassium removal by corn plants grown in undiluted soil

Corn plants were grown in undiluted soil from the several fertility experiment plots indicated in table 12. The soil used in this test was from the portions of the plots that have received additions of limestone. Triplicate pots containing 3000 gm. of soil were planted June 3 and harvested July 25. Five plants were started in each pot and at the end of the first week the plants were thinned to two.

Additions of acid phosphate, potassium chloride, and sodium nitrate were made to certain pots as indicated in the table 12. The quantities of these materials per pot were as follows: Acid phosphate 0.9 gm.; potassium chloride 0.375 gm.; sodium nitrate 0.357 gm. These quantities are equivalent to 300 p.p.m. of acid phosphate, 125 p.p.m. of potassium chloride, and 119 p.p.m. of sodium nitrate. Sodium nitrate and potassium chloride were added in solution, and acid phosphate was mixed with the soil 10 days before planting the corn. Triplicate tests of each soil were made and the results averaged for the three pots.

The object of this test was to determine to what extent corn plants grown for a more extended period than the plants in previous tests would reflect variations in potassium supply brought about by fertilizer treatment, especially by that not including potassium. The additions to the pots provided for a supply of available nutrients other than those with which the plots had been fertilized.

The weights of plants on the several soils are not widely different with the exception of those on the forestry plot soil, which exhibited a high productive power whenever it has been used in the pot tests with wheat seedlings. With additions of phosphorus, potassium, and nitrogen to the soils in the pots just previous to planting the corn, there were, however, some increases in weights of plants produced. The addition of potassium chloride to the soil from a plot fertilized with acid phosphate caused an increase, whereas the addition of sodium nitrate with potassium apparently adversely affected the growth, resulting in a plant weight equal to that on the soil with no addition. The potassium removed, however, was more than that of plants grown in the same soil without additions.

Regardless of the effect on weight of dry matter produced, potassium chloride invariably caused an increased removal of potassium, as well as a greater concentration than in plants grown on soil receiving no addition of available potassium. The increased amount of potassium taken up by plants grown on soil that had been fertilized with muriate of potash also reflects the larger available supply, although the yield of dry plant material did not differ materially from that of plants on unfertilized soil.

In this comparison of soils from the several fertility plots no check with sand only was included. But a check on that portion of the potassium removed that could have been obtained from the seed is furnished by available

data regarding the weight and potassium content of an average sample of seed planted in this and in the previous test with corn. The average weight of the corn was 0.2552 gm. per kernel, and the potassium content per gram of seed was 0.003 gm., so that 2 seeds supplied 1.5 mgm., a negligible quantity in comparison to the total amount removed. These corn plants grown in undiluted soil have assimilated varying amounts of potassium, which quite consistently indicate differences in the available supply.

SUMMARY

Experiments have been in progress to ascertain whether potassium abstracted by plants will furnish reliable information concerning the available supply in soils.

Neubauer's seedling method of soil analysis, in which a large number of plants are grown in a small amount of soil diluted with sand, has been employed.

The soils used in measuring the availability of potassium by means of plant seedlings included those from fertility experiment plots that have been fertilized and under observation for a period of years.

Several series of tests were made varying somewhat as to the soil dilution with sand, the number of plants, and other conditions.

From a comparison of rye and wheat, it was found that the latter produced a larger weight of plant material and removed more potassium.

It was concluded from experiments with varying proportions of soil and sand, and with varying numbers of plants, that 100 wheat seedlings grown in 200 gm. of soil diluted with 1000 gm. of sand furnished the optimum conditions.

A test with wheat and buckwheat plants grown for 20 days showed that buckwheat abstracted more potassium.

Additions of available nutrients to soils that have been fertilized and cropped for many years affected the potassium removal.

It is apparent that the amount of potassium removed is closely associated with increased plant growth produced by supplying available nutrients.

Additions of available potassium had a marked influence in stimulating the growth of wheat seedlings.

From the increased potassium removed by wheat seedlings grown on soil that have received applications of potassium in fertilizers and manure, it is evident that a potassium residual from that applied is readily available.

Potassium abstracted by wheat seedlings from limed soil that has received no other fertilizer than acid phosphate for over 30 years, does not indicate the same depletion of available potassium as do a dilute acid extraction of the soil and decreased corn yields during recent years.

The potassium content of corn plants grown beyond the seedling stage in undiluted soil varied very consistently with the available supply, as indicated by the fertilizer treatment and the additions to pots.

REFERENCES

- (1) HALEY, D. E. 1924 A biological measurement of the availability of potassium in soils. Penn. Agr. Exp. Sta. Bul. 188.
- (2) NEUBAUER, H., AND SCHNEIDER, W. 1923 Food consumption of germinating plants and their use in the determination of the fertilizer requirements of soils. *Ztschr. Pflanzener nähr. u. Düngung*. 2 (A): 329-62.
- (3) ROEMER, T. 1925 Test of the Neubauer method. *Zuckerrubensbau*. 7 (1).
- (4) THORNE, C. E. 1924 The maintenance of soil fertility. Ohio Agr. Exp. Sta. Bul. 381.

PLATE 1

EFFECT OF AMMONIUM SULFATE AND SODIUM NITRATE ADDITIONS ON GROWTH OF WHEAT SEEDLINGS

Pot 28, ammonium sulfate; pot 27, sodium nitrate

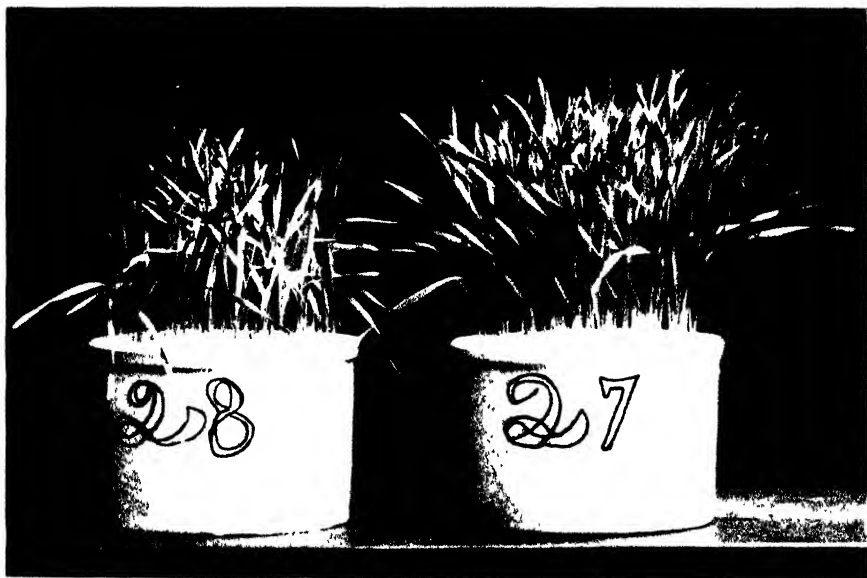


PLATE 2

EFFECT OF LIMING ON THE GROWTH OF WHEAT SEEDLINGS

FIG. 1. Unlimed soil — Pot 2, mono calcium phosphate, pot 21, potassium chloride, pot 53, ammonium sulfate, pot 45, sodium nitrate

FIG. 2 Limed soil — Pot 3, mono calcium phosphate, pot 23, potassium chloride, pot 54, ammonium sulfate, pot 46, sodium nitrate



FIG. 1



FIG. 2

PLATE 3

GROWTH OF WHEAT SEEDLINGS ON SOILS FROM 5 YEAR ROTATION PLOTS

FIG. 1 Pot 61, unfertilized soil, pot 65, plot 2, acid phosphate.

FIG. 2 Pot 69, plot 3, muriate potash, pot 73, plot 5, sodium nitrate

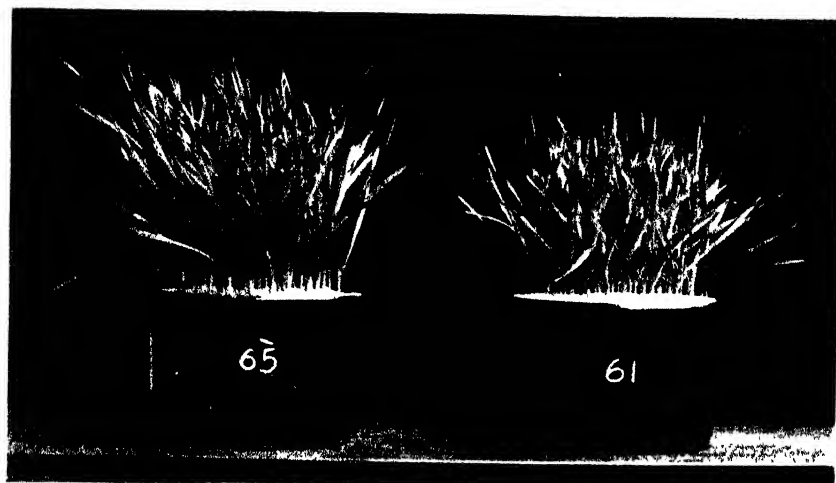


FIG. 1



FIG. 2

PLATE 4

GROWTH OF BUCKWHEAT AND WHEAT IN SEVERAL SOIL DILUTIONS

FIG. 1. Buckwheat—Pot 1, sand; pot 2, 50 gm. soil; pot 3, 100 gm. soil; pot 4, 200 gm. soil; pot 5, soil, no dilution

FIG. 2. Wheat—Pot 11 sand; pot 12, 50 gm. soil; pot 13, 100 gm. soil; pot 14, 200 gm. soil; pot 15, soil, no dilution

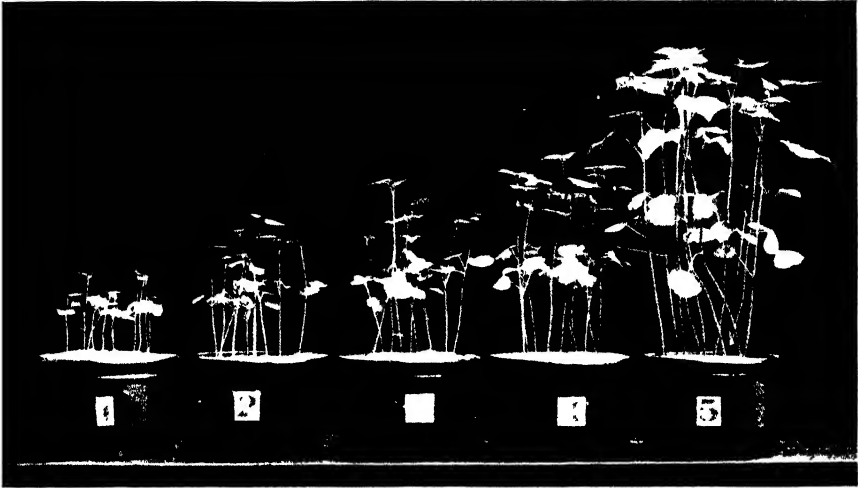


FIG. 1

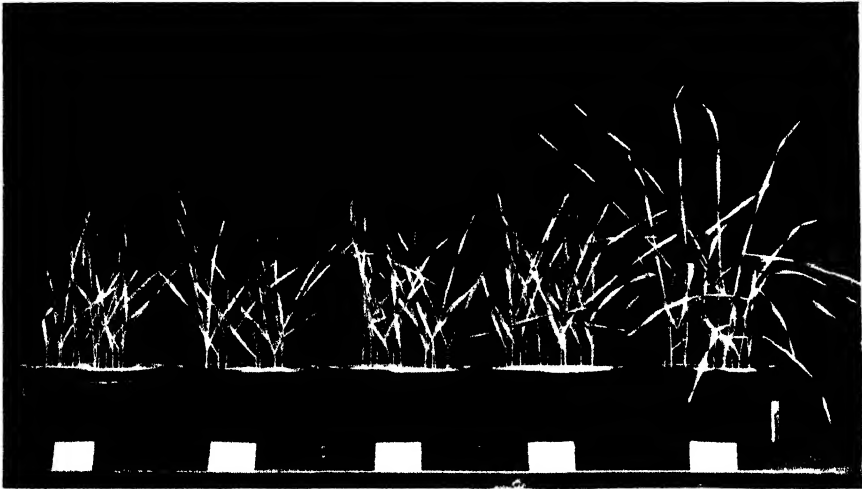


FIG. 2

EFFECTS OF SOME ELECTROLYTES ON KAOLIN AND THE PROBABLE RELATION TO THE SOIL

JOHN R. SKEEN

University of Pennsylvania¹

Received for publication May 28, 1926

In a previous paper (22) evidence was presented indicating that the formation of hardpan in clay soils was a function of the pH. In soils observed in the vicinity of Philadelphia, the clay particles forming the hardpan are bound either by iron or aluminum, as the oxides or silicates. They present a layer that is practically impermeable to water and are a factor in local plant distribution, because, severed from the connection with the subsoil, they can dry out more thoroughly and thus cause a higher osmotic pressure, and, it was believed, because iron and aluminum were in the soil solution in sufficient quantities to be toxic to some plants. In a recent article Magistad (15) contributed toward the toxicity problem. This paper deals with a preliminary physical chemical consideration of the behavior of kaolin in an effort to explain how hardpans are formed in acid clay soils.

NATURE OF THE HARDPAN

The hardpan in question occurs in silt and clay loams which have become acid. It is found as a red or silvery layer at various distances below the surface, but is most common at depths of 6 to 20 inches. The layer is made up of clay aggregates more or less cemented by the silicates and oxides of aluminum and iron. When dry, the layer is exceedingly hard, and, soaked in water, becomes tough. In both conditions it is impermeable to water so that the surface soil, even a short time after a rain, loses most of its capillary moisture, while the subsoil immediately below the layer generally has a maximum supply.

In the Philadelphia region, where this hardpan occurs, no succulents survive the summer months.

The surface 6 inches of the soil is generally coarsely granular and dry and relatively free of organic matter. When saturated it tends to puddle and is most unsatisfactory to work.

ADAPTABILITY OF KAOLIN FOR EXPERIMENTS

Several methods of fractionating soils were tried but the same type and size of particle was not consistently obtained. Although, in all the soils used,

¹Department of Botany.

when well washed in distilled water, the particles migrated toward the anode in an electric field, yet they migrated at different rates in samples of soil prepared in the same way at different times. Results obtained with one sample could not be compared with observations made on a different batch of what was apparently the same material. As reproducible results are imperative, it is necessary that the material be of a uniform quality. Soil samples proved impractical and as a substitute, the kaolin supplied by Merck and Company was used. It is fairly uniform in texture and chemically is nearly free of everything except HCl.

It is believed that any conclusions that may be drawn when kaolin¹ is used as the medium are applicable to "clays" as a class. According to Washburn (27), one of the most common mineralogical constituents of "clay" is kaolin, which in its purest form is a crystalline mineral having the composition $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$. Warrington (26) states that "in a typical clay (kaolin) derived from the decomposition of feldspar, the whole substance has practically the same chemical composition. . . ." Merrill (18) says, "orthoclase is one of the commonest and most abundant of the feldspars and forms an essential constituent of acid rocks. . . . As a rock constituent, the potash feldspars are of primary importance yielding on their decomposition . . . the material kaolin. . . ."

According to Hall (6) and Hilgard (9) orthoclase may be written $\text{K}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{SiO}_2$. It decomposes in carbonated water forming kaolinite, $\text{Al}_2\text{O}_3(\text{OH})_4 \cdot \text{Si}_2\text{O}_5$, the basis of clay. Swanson (23) gives another formula for kaolinite and graphically writes it differently from other authorities. It has been suggested that kaolin may be a loose Van Bemmelen (24, 25) adsorption compound of aluminum oxide and sand which may behave as a unit although it is not necessarily one.

The structure of kaolin seems doubtful but it is an important constituent of nearly all clay soils.

METHODS

Determinations were made simultaneously on the same sample of kaolin for:

1. Turbidities at intervals up to forty minutes,
2. Final volumes of the sedimented kaolin at various intervals up to two days,
3. Conductivity, always comparing to blanks containing no kaolin,
4. pH,
5. Sign and intensity of the charge on the particles.

The kaolin was put into collodion bags and dialyzed in a stream of running water for eight weeks. The conductance of the supernatant liquid in which the kaolin was kept was only slightly lower than that of distilled water exposed the same length of time although protected from dust. It was believed that the kaolin so prepared was "chemically pure" and that it did not ionize appre-

ciably. Kaolin was later prepared in an electric dialyzer (17), and washed several times by decantation. So prepared, its resistance is practically the same as that of freshly distilled water. A stock was thus easily prepared.

Determined stock solutions of aluminium chloride, calcium chloride, barium chloride, sodium chloride, hydrochloric acid, and sodium hydroxide were made up and the same solutions were used throughout the work. The sodium hydroxide solution was redetermined from time to time. The stock solutions were diluted with distilled water to give the required concentrations. The concentrations of the other solutions used were only approximately accurate.

A large number of 50-cc. graduated cylinders were procured and twenty chosen having the same internal depth, so that the internal friction in any check experiments would be the same through any given time interval. By means of a pipette, 2 gm. of kaolin were put into each of these graduates, and made up to the 50-cc. mark with the proper solution. In all cases two graduates with kaolin and distilled water were prepared as a check. The graduates were then tightly stoppered by inverting slightly oversize, pliable rubber stoppers into the openings. In this way the stoppers made a flat surface entirely across the mouth of the cylinders. This prevented kaolin from sticking to the much greater surface exposed by variously tapering stoppers. It was assumed that the same quantity of kaolin adhered to the stoppers in all cases. An error was introduced, but a constant one well within the limit of accuracy demanded by the experiments.

1. Turbidities as determined in these experiments are entirely comparative. The standard of comparison chosen was distilled water. Six concentrations of three electrolytes were generally prepared simultaneously when strictly comparative data was wanted. The kaolin was thoroughly shaken in the graduates three or four times at intervals before observations were made. Turbidities were compared at 2, 4, 7, 10, 15, 20, 25, 30, and 40 minutes and, when necessary, longer. The graduates were placed in front of a bank of 25-watt lamps. Black paper was placed behind the graduates so that a good contrast could be had under uniform conditions. After some little practice, it was possible to determine by simply observing turbidities, not only the concentration of the solutions but which solutions were in any given cylinder.

2. The volumes occupied by the kaolin in falling were determined at the same intervals as the turbidities and also at 12, 24, and 48 hours. The bulk of the kaolin settled together leaving a more or less dense supernatant liquid.

3. When the liquid was entirely clear, it was pipetted out and put into a tall, narrow beaker having a capacity of about 30 cc. The resistance in ohms of the liquid was recorded. The resistance in ohms of the same concentration of the liquid before determined, treated in the same way but with no addition of kaolin, was obtained. This gave an immediate and exact comparison of the degree of adsorption or non-adsorption of the electrolyte by kaolin.

4. Determinations for pH were made by means of the potentiometer using

a saturated calomel cell. With the unbuffered solutions, results were entirely unreliable and only those results are recorded which are dependable.

5. Migration measurements were made by means of the apparatus recommended by Northrop (21). The cell used had a depth of 0.2 mm. The particles were observable under the low power of the microscope and migrated at a fair rate in an electric field of 110 volts and 0.3 amperes. The field of the scope was graduated and a graduated glass disc was inserted just beneath the eye piece. By means of a stop watch it was then possible to determine the time required for particles to move a given distance. No observations were made on particles within 55μ of the upper and lower surfaces of the cell. Thus starting at a depth of 55μ from the top of the cell, determinations were made at intervals of 10μ at 10 depths. As many determinations of the rate of movement of the particles as possible were made at all the depths mentioned with the same sample. Because of the rapid settling of the particles, very few determinations could be made in some solutions at the upper levels. In such cases samples were made until the speed of at least ten particles could be observed.

The poles were changed at intervals and the rate of migration noted in the opposite direction. The mean rate of all depths of three or more separate experiments are recorded for each concentration used. As the particles are far from spherical and as in some solutions they form aggregates whereas in others they are very much dispersed, no attempt to calculate the intensity of the charge was made. In all cases the rates recorded are relative to the rate of migration of particles in distilled water.

The figures recorded in table 1 are believed dependable for the weaker solution, but are not satisfactorily reproduced at concentrations of 0.1 *M* or more. Particularly is this true in the case of HCl.

RESULTS

The results reported in table 1 are believed typical for kaolin treated as described. The figures for resistance are the mean of many determinations made on at least three separate check experiments. Final volume results are the mean of two or more readings on at least five check experiments. This also applies to the data on turbidity. It was hoped that pH determinations would be sufficiently accurate to indicate the possibility of the selective adsorption of ions by kaolin. As no reasonable checks were obtained, only the pH limits of the solutions of salts are recorded.

1. It was found that the effects of different solutions on the quantity of kaolin suspended were most apparent 15 minutes after the last shaking. As the bulk of the kaolin settled in the graduates there remained suspended sufficient kaolin particles to color the liquid. A liquid such as 0.001 *M* Al cleared immediately after shaking. The "turbidity" of this solution is recorded as 0. Less clear solutions were arbitrarily assigned a number as they

TABLE 1

MOLAR SALT CONCENTRATION	OHMS - KAOLIN	OHMS + KAOLIN	VOLUME OF SEDI- MENT AT 24 HOURS	TURBIDITY AT 15 MINUTES	TURBIDITY AT 30 MINUTES	SPEED PER SECOND	pH
Water	80,000	13,500	cc. 7.30	7	2	μ -14.5	8.4
NaOH	1.0	6	5.94	5	1	-12.9	
	0.3	13	5.58	6	1	-14.5	
	0.1	41	5.67	7	2	-16.7	
	0.03	114	6.75	7	3	-20.5	
	0.01	396	6.93	14	6	-26.0	
	0.003	1,755	6.39	28	12	-28.0	
NaCl	1.0	12	6.25	10	2	-10.4	8.4
	0.3	25	5.72	9	3	-23.3	8.5
	0.1	76	6.24	9	3	-24.5	
	0.03	232	7.34	8	2	-21.6	
	0.01	716	7.44	7	2	-21.6	
	0.003	1,912	7.45	6	2	-20.2	
HCl	1.0	3	5.58	7	1	+16.0	
	0.3	7	6.00	6	1	+	
	0.1	22	6.84	2	1	-	
	0.03	61	7.59	2	1	-	
	0.01	205	7.23	3	1	-	
	0.003	600	6.78	4	1	-11.3	
CaCl ₂	0.6	12	5.86	9	1	+8.9	7.5
	0.1	45	6.30	4	1	-5.7	8.5
	0.03	132	6.43	3	1	-7.1	
	0.01	450	6.68	3	1	-8.6	
	0.003	1,080	6.68	4	1	-10.2	
	0.001	3,040	6.76	5	1	-10.3	
BaCl ₂	0.0003	8,800	6.86	6	1	-10.8	2.07
	0.6	10	5.76	9	2	+3.0?	
	0.1	44	6.35	6	1	-4.5	
	0.03	120	6.61	4	1	-	
	0.01	376	6.61	2	1	-7.7	
	0.003	1,020	6.53	3	1	-10.8	
AlCl ₃	0.001	3,340	6.53	4	1	-12.9	8.00
	0.0003	7,950	6.26	6	1	-15.0	
	0.6	7	6.52	11	4	+6.9	
	0.1	21	7.94	8	3	+19.2	
	0.03	62	8.86	8	3	+29.5	
	0.01	190	8.90	8	3	+29.5	
	0.003	433	9.92	7	2	+32.0	
	0.001	1,210	10.74	0	0	+21.2	
	0.0003	3,040	8.02	2	1	-12.3	

increased in turbidity. At 15 minutes from shaking, the graduate containing 0.3 *M* NaOH was less turbid than the graduate containing water. The value 6 is assigned for the turbidity of 0.3 *M* NaOH and 7 for kaolin and distilled water. The turbidity value for water is kept constant for the comparison of check experiments.

The turbidity value of kaolin in water after the 30-minute interval is placed at 2, and perfect clarity of the supernatant liquid at 0.

It is then clear that in all the solutions recorded in table 1 (except NaOH) the particles of kaolin settle more or less rapidly. It is also evident that different concentrations of the same electrolyte affect turbidities to a marked extent. With HCl, CaCl₂, BaCl₂, and AlCl₃ there is a concentration producing the least turbidity. Any increase or decrease from this concentration results in an increase in turbidity. It is significant to note that the least concentrated solution of NaCl produces the clearest solution, whereas the most concentrated solution of NaOH produces the least turbidity.

Al is the most efficient ion in clearing solutions of kaolin, followed by Ba, Ca, and H in the order given. With turbidities as criteria, the efficiency of the ions in clearing solutions reduced to simplest terms if H-1, Ba-3, Al-100. Ca is less efficient than Ba in this regard.

NaCl and particularly NaOH, greatly increase the turbidities of the solutions. Even traces of NaOH have a marked effect on kaolin, keeping quantities in suspension for two hours or longer.

Sodium sulfate behaves as NaOH, greatly increasing the turbidity of the solutions. Sodium citrate, even in concentrations of 0.001 *M*, causes the greatest increase in turbidity and prevents clearing for 10 days and sometimes longer.

Sucrose and urea have no effect on turbidities in weaker concentrations. The increase in the specific gravity of the more concentrated solutions tends to keep the particles in suspension for a slightly longer time than distilled water. Thus non-electrolytes have no effect on the clarity of the supernatant liquid.

These results are entirely as could be predicted and are used only as checks on the effect of ions of different speed and valency and as criteria of the relative degree of disassociation of the kaolin aggregates.

2. The concentrations of less than 0.03 *M* Na and H have approximately the same aggregate result, that is, little changing of the final volume of the sediment as compared to water. The OH ion decidedly decreases the final volume of the sediment as do the Ca and Ba ions. The SO₄ ion has a marked reducing effect on the final volume of the sediment whereas the tri-valent citrate ion reduces the volume by almost 50 per cent.

Of the ions used, Al alone increases the volume of the sediment to a marked extent.

The more concentrated solutions of all the salts used decrease the final

volume of the sedimented kaolin very considerably either compared to water or to the less concentrated solutions of the same salt.

Sucrose and urea had no affect whatever on the volume of the sediment produced, the values obtained in all concentrations being the same as those obtained with water. Having no tangential value, the curves are not drawn.

From the curves of figure 1, it may be seen that the volume occupied by 2 gm. of kaolin may be only 60 per cent of that volume attained in water, or it may have increased 50 per cent. Microscopic examination shows that such great differences are due to the formation of sizable aggregates in the case of the greatest increase in the final volume of the sediment, and to the complete break-up of the aggregates when the final volume is markedly decreased.

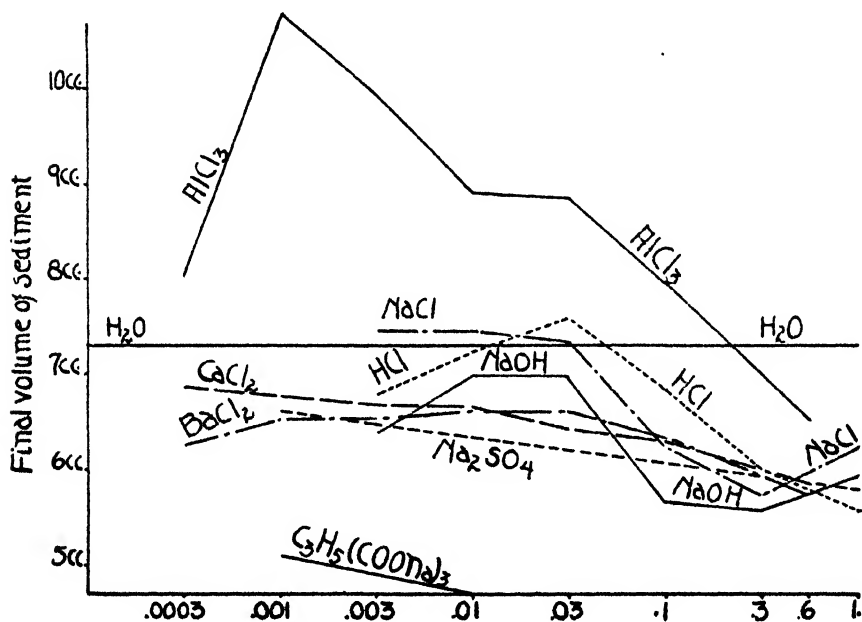


FIG. 1. FINAL VOLUME OF SEDIMENTED KAOLIN IN GRADUATES AT END OF 24 HOURS AFTER SHAKING WITH MOLAR CONCENTRATIONS OF ELECTROLYTES INDICATED

In a general way, it may be said that the more turbid is the supernatant liquid, the less will be the final volume of the sediment.

The experiments on turbidities and sediment volumes furnish a fair check on each other, and are important to this paper only as they indicate that the degree of aggregation of the kaolin particles can be markedly altered, that active anions can break up the aggregates—a tri-valent anion most completely, and a di-valent anion to a greater extent than the OH ion. Of the cations used, Al and Na alone cause the flocculation of the particles.

3. Figures 2 and 3 show that in the weaker solutions of the electrolytes (HCl excepted) no adsorption of the electrolytes occurs. On the contrary,

the solutions apparently become more concentrated in the presence of kaolin. These findings are in accord with those of many investigators working with substances other than kaolin, and again demonstrate that the Freundlich adsorption isotherm does not hold for dilute solutions.

No comparison of adsorption rates of the electrolytes can be made by this method, insofar as adsorption occurs only in the more concentrated solutions where the conduction is too great for accuracy. However, the method furnishes a good comparison of the "negative adsorption" of the salts. This seems to be a false term, for in such solutions something must be adsorbed—if not the solute, then the solvent. However, the following "negative adsorption" sequence may be written: $\text{NaCl}, \text{NaOH} > \text{BaCl}_2 > \text{AlCl}_3, > \text{HCl}$, in which HCl is adsorbed at all concentrations.

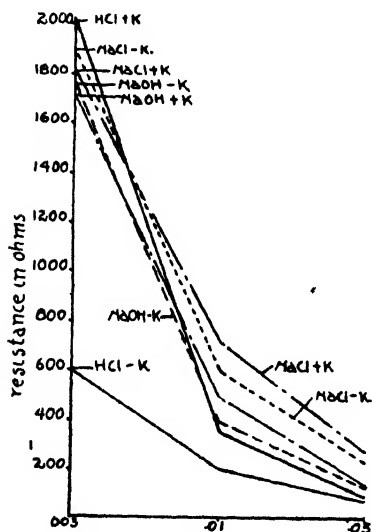


FIG. 2. RESISTANCE IN OHMS OF SOLUTIONS WITH AND WITHOUT KAOLIN AT VARIOUS MOLAR CONCENTRATIONS

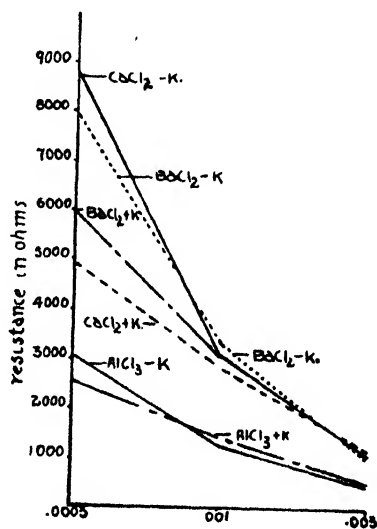


FIG. 3. RESISTANCE IN OHMS OF SOLUTIONS WITH AND WITHOUT KAOLIN AT VARIOUS MOLAR CONCENTRATIONS

It is significant to note that adsorption occurs in weaker solution of Al than of Ba and Ca . A fairly concentrated solution of Na must be present before it is adsorbed by kaolin.

4. The pH of kaolin when dialyzed as described is slightly greater than 8.4. Dilute solutions of the chlorides of Na , Ba , and Ca have little effect on this value. Increased concentrations of AlCl_3 rapidly decrease the pH of the kaolin solution but as the curve obtained is very irregular and is believed to be unreliable, it is not recorded. The few pH values reported were checked innumerable times and consistently gave the same figures. They are believed to be reliable, and are accurate within quite narrow limits. Ba behaves practically as Ca .

5. Cataphoretic measurements are reported in microns per second. The accuracy of the values given depends entirely on the number of determinations made at the different levels. The greater the number of determinations, the less is the chance of error and the closer is the approach to an absolute comparison. Most of the figures given represent an average of more than 100 observations at 10 levels from three or more separate samples. Comparative results are reproducible within narrow limits, but there is difficulty in obtaining specific values.

A considerable error is obtained in the more concentrated solutions, because

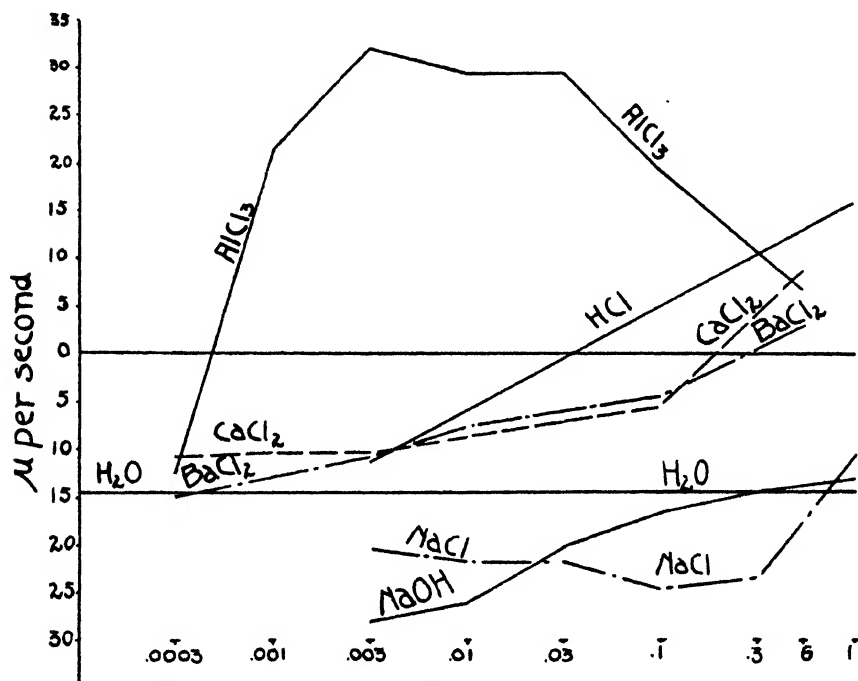


FIG. 4. RATE OF MIGRATION OF KAOLIN PARTICLES IN $\pm \mu$ PER SECOND WHEN EFFECTED BY MOLAR CONCENTRATIONS OF ELECTROLYTES

of the physical nature of the electrolyte in retarding the particles. The true speed of the particles is believed to be somewhat decreased. Then, too, the size of the particles varies in different solutions and the mass of the particles introduces an error. It was only with the greatest difficulty that any figures at all could be obtained for HCl. This acid appears to neutralize the charge of the particles very completely, causing no motion whatever in most cases, although the particles remain suspended. It was clearly seen that in the same solutions at any given level, particles migrated to both the anode and cathode. Indeed the same particle could change the sign of its charge several times before passing from the field of the microscope.

As before mentioned, it is impracticable to calculate the intensity of the charge because the particles have no definite size or shape and they may form aggregates of varying complexity. The intensity of the charge is here measured by the speed of migration of only those particles which move. This introduces still another error in observations made on slow moving particles. Results are comparative and are presented only as such.

Figure 4 illustrates the effects of the various ions on the charge of the particles. It is evident that NaOH increases the charge to the greatest extent in the negative direction and that NaCl does not have quite so pronounced an effect. Ba, Ca and HCl decrease the charge and in dilute solutions, as does Al. But Al rapidly neutralizes the charge and even in dilute solutions reverses it to a marked extent. Only the most concentrated solutions of Ca and Ba reverse the charge of the particles, and HCl is more efficient in this regard than Ca or Ba. Increasing the concentrations of NaOH and NaCl decreases the charge of the particles. But this effect, as before pointed out, may be due to the internal friction of the solutions. When the concentration of Al is increased beyond 0.003 *M*, the charge is also decreased.

It was thought that some correlation between the intensity of the charge on the particles and the final volume of the sediment could be obtained from these experiments. That the degree of flocculation is independent of the sign or intensity of the charge is apparent from a comparison of the curves of figures 1 and 4. On the contrary, with Al, it seems that as the charge increases, the final volume of the sediment increases, but this statement does not hold for the other electrolytes. This condition is at variance with suspensions of the metals where the intensity of the charge determines the amount and speed of precipitation. There does seem to be some correlation between the intensity of the charge in either direction and the turbidity of the solution. Table 1 shows this quite clearly. It appears that flocculation with kaolin depends on the quantity and quality of the supernatant liquid. Calcium acts as a deflocculating agent on "chemically pure" kaolin as compared with the final volume of the kaolin in distilled water, although it decreases the charge on the particles. This statement is quite at variance with the generally accepted view.

DISCUSSION

Calcium is generally regarded as a flocculating agent in soils. The data presented indicate that it is a deflocculating agent on kaolin. There is no discrepancy in the two statements when it is considered that this paper deals with high resistance kaolin. Mattson (16) in discussing this question is of the opinion that calcium acts as a binder, or bridge between two particles of clay, holding them together. The number thus held increases with the pH, in this way hastening flocculation of the soil in question. It is believed that any effect that Ca may have as a flocculating agent is due only to the reduction in the effect produced by some electrolyte already present which has

deflocculated the soil to a greater extent than calcium is capable of doing. There is also the possibility of the precipitation of the hydrates of aluminum and iron, in this way binding together many clay and sand particles. Then too the flocculating effect of calcium on soils probably is not due directly to the effect on the soil particles but indirectly by the precipitation of silicates and humates.

The cataphoretic measurements reported by Dayhuff and Hoagland (5) in working with clay suspensions show the same general tendency as those given here. They say, in part,

within the range of reaction found in the soil solutions, the clay colloid . . . showed no change in the sign of its electric charge, and the nature and concentration of the cations present in the solution had a predominant influence on the stability of the colloidal suspension.

The observations of Dayhuff and Hoagland are in harmony with the present findings, with the single exception that 0.8 milliequivalents of aluminum as the chloride show a pronounced positive charge with kaolin. This is believed due to the greater purity of the kaolin used over the clay suspension of Dayhuff and Hoagland.

As before pointed out, HCl is adsorbed at all concentrations used and the nature of the curve indicates that HCl would be adsorbed even at infinite dilution. No other electrolyte used was adsorbed at dilute concentrations, but were "negatively adsorbed." The data presented indicate the "negative adsorption" sequence: NaCl, NaOH > Ba > Ca > Al.

The pH of the $AlCl_3$ solutions is very low. The hydrolysis of this salt would result in the liberation of a strong acid and a very weak base. It is then possible that it is the HCl which is adsorbed and not the molecule of the salt. The pH of the chlorides of calcium and barium is much higher, and adsorption does not begin until the solutions are comparatively concentrated. The hydrolysis of NaCl would result in the liberation of an equally strong base and acid, and as demonstrated by the curves, NaCl is least adsorbed by kaolin. It is unfortunate that pH determinations were not sufficiently accurate to indicate consistently the adsorption of the acid. Interpreted in this way, however, adsorption results are in accord with the work of Miller (19). In working with sugar charcoal, he comes to the conclusion that strong inorganic bases are "negatively adsorbed" and that adsorption from solutions of some salts is exclusively hydrolytic in nature, whereas from others, it is partially hydrolytic and partially molecular. Such conclusions are in accord with the theorem of Gibbs, that substances lowering the surface tension of a liquid are positively adsorbed at an interface, whereas those raising the surface tension tend to keep from the surface. This "negative adsorption" effect does not increase with the concentration. HCl decreases the surface tension of water whereas the other electrolytes used increase it. The work of Miller seems sufficient to explain the curves of figures 2 and 3.

In this regard, it is suggested that the theories of Harkins (7) together with the fixation effects of Langmuir's (11, 12, 13) secondary or residual valencies furnish some explanation of the phenomena of adsorption by kaolin. However, the data here presented are not sufficient to test their applicability.

From the above discussion, it would seem that adsorption is a function of pH and, as Loeb (14) has pointed out in his work on gelatine, when the pH is kept constant, there is no Hofmeister series. That his observations do not apply in the case of kaolin is apparent from figures 1 and 4. There can be no correlation between pH and flocculation when the curve for HCl is compared with any of the others, the flocculating tendency is the same, but the degree is vastly different. In the migration of the particles, neither the degrees nor the tendencies are similar.

There can be little question that each ion has its own specific effect on the particles with regard to degree of flocculation or deflocculation. That this effect is produced in the most dilute solution used is demonstrated by the curves of figure 1, despite the fact that the electrolytes used (HCl excepted) are "negatively adsorbed" at those concentrations.

The writer knows of no explanation that can adequately account for the phenomena described and only ventures the suggestion that Gibbs' Theorem is operative as described by Harkins and Miller, and that the effects of specific ions are due to their fixation to a greater or less extent at the surface because of the secondary of residual valencies described by Langmuir.

Figures 2 and 3 show conclusively that in the salt concentrations found in the soil, that is, concentrations of less than 0.003 *M*, the essential ions are not adsorbed by the particles of kaolin. At least, if any adsorption occurs at these concentrations, it is a negligible quantity. As Bouyoucos (2) has pointed out, this fact is opposed to the prevalent notion. He states in part,

experimental evidence . . . proved the opposite view, namely, the solution around the soil particles and in the very fine capillary spaces is less concentrated than the mass of the solution. All experimental evidence obtained is overwhelmingly in favor of this view.

SOLUBLE AND AVAILABLE ALUMINUM IN THE SOIL

From the data presented, it is clear that particles of clay are in a partially deflocculated condition at neutral pH and are progressively deflocculated as the pH increases. This statement is believed to apply only to soils with a high clay content. This is the only conclusion tenable in view of the successive solubilities of the mono-, di-, and tri-valent bases with decreasing pH. Another way of stating the condition, is that the soil is progressively flocked with increase in acidity. This flocculation, as before pointed out, is not due to the H-ion but rather to the ions brought into the soil solution as a result of the increasing acidity. It follows then that aluminum and iron are present in the soil solution in an easily soluble form in significant quantities at pH below 5.00.

In an effort to demonstrate that aluminum was present in significant quantities in solution at pH of from 4 to 5, the following experiment was performed.

Stock solutions were prepared and determined and the following nutrient solutions were made by dilution:

Ca (NO ₃) ₂	0.016 and 0.008 M
MgSO ₄	0.008 and 0.004 M
H ₃ PO ₄	0.02 and 0.01 M
Al ₂ (SO ₄) ₃	0.002 and 0.001 M

To change the pH, a liter of the above nutrient was titrated with standard KOH. The curves are of exactly the same type as Clark (4) found in the titration of H₃PO₄ with normal KOH. The greatest value of the tangent is reached between pH 3.8 and 5.2. The exact amount of Al added per liter being known, analyses were made to determine the amount precipitated at various acidities. Insofar as no Fe was added, Al determinations could be made directly after separating off the Mg and Ca. To lose as little Al as

TABLE 2
Effect of dilution on Al solubility

pH OF NUTRIENT	Al IN SOLUTION	
	2 × concentration	× concentration
	p.p.m.	p.p.m.
4.35	6 0	6.5
4.53	3.7	4.0
4.70	2 3	2.1
4.85	0 9	...
5.42	1.1	...

possible, the volumes were kept down to less than 75 cc. and precipitates were washed with as little water as possible, tests being made for Ca and Mg constantly. The pH of the precipitating reactions were kept below 7.0.

Analyses of the precipitate formed showed in both series practically the same amount of Al in solution. From table 2 it may be seen that dilution has no apparent affect on Al solubility.

The Al in solution could not possibly have any effect on the soil or kaolin particles with regard to their flocculation. Even if the method of analysis employed is assumed to give 100 per cent error, the quantities of Al in solution could cause no flocculation of the kaolin.

A search for the reason brought to light several articles that had been overlooked. A comparison of the determinations made by Magisted (15) for Al present in solution indicates that the quantity of soluble Al in solution is a function not only of pH but of PO₄ as well. Crone's nutrient, high in phosphoric acid, renders Al more insoluble, whereas the nutrient of Hartwell and Pember with only a small molar concentration of phosphoric acid equiva-

lent, increases the solubility of Al over that found in the nutrient reported in this paper.

Whether this view is the correct one can only be determined by further work. However, the experiments of Mirasol (20) indicate that this opinion is true. He found that the addition of acid phosphate reduced the toxicity of Al to plants and that the decrease in toxicity depended on the rate of acid phosphate application. Hartwell and Pember (8) observed that the amount of active Al is decreased by the increase of acid phosphate application despite the increase in the acidity of the soil so treated. From the determinations of Burgess (3) and Mirasol it seems that aluminum is present in the soil at low pH, not as the phosphate (unless phosphate was added) but as the nitrate, sulfate, or chloride. From their data it also seems that the quantities present depend on the nature and antecedents of the soil.

In order to obtain some estimation of the quantity of aluminum and iron present in the solution of the soil before reported upon (22) in connection with the formation of hardpan, the method employed by Blair and Prince (1)

TABLE 3
Analysis of 1 liter water extract from four soil samples

SOIL SAMPLE	pH	COMBINED ALUMINUM AND IRON
		<i>p.p.m.</i>
1	4.5	52
2	4.75	45
3	5.0	5
4	5.2	12

was used. Table 3 shows the results obtained by the analysis of 1 liter of the water extract obtained from 5 kgm. of four soil samples.

Two samples were taken from the southern part of the area in which hardpan was found, the pH being 4.5 and 4.75; and two samples were taken from the part of the area where hardpan was not found, the acidities being 5.0 pH and 5.2 pH.

The southern part of this area is greatly affected by quantities of smoke from neighboring factories and railroads.

This is believed to be the proof needed to support the theory of hardpan formation to be advanced. A comparison with the results of Blair and Prince show these values to be considerably higher than they obtained. Furthermore, soils 3 and 4 do not fall on a theoretical curve that can easily be imagined. It is probable that this is because of different quantities of phosphate present in those soils.

A THEORY FOR THE FORMATION OF HARDPAN IN ACID CLAY SOILS

The theories advanced relative to the formation of such hardpans have been based almost entirely upon the mechanical behavior of the soil parti-

cles—surface drying, precipitation of various cations because of supersaturation, the carrying down of small particles, and so on. These factors undoubtedly play a part, but in themselves are not sufficiently in accord with the facts observed in the case. Some years ago Warington (26) observed:

Hydrated ferric oxide is another colloid substance which in sandy soils undoubtedly plays the part of a cementing agent. In rocks and soils of the Red Sandstone formations, its influence is plainly marked. Perhaps, however, the most obvious example of the cementing action of ferric oxide is afforded by the formation of iron-pan in Moor soils. . . . The iron has been dissolved out of the surface soil by the action of humic acids . . . passes in solution into the subsoil where it is precipitated, with the result that the sand at a certain depth is cemented together and an iron-stone produced.

Warington had little evidence in support of this view other than observation and did not mention that the same phenomena could obtain in clays as well. The evidence presented here supports his view and attempts to explain just how that layer comes into being.

It has been shown in the weak concentrations found in the soil solution that the essential salts necessary for plant growth are kept away from the surface of the clay fraction. This is in accord with the Second Law of Thermodynamics and the Theorem of Willard Gibbs. That the effects of the various cations in these concentrations, despite apparent non-adsorption, is evident has also been shown. The explanation suggested is that the residual valencies induce a weak chemical attraction such as Langmuir pointed out. HCl is greatly adsorbed by kaolin and according to Miller, this applies to other acids in the case of sugar charcoal. As shown in final volume of sediment curves, both HCl and Na_2SO_4 greatly deflocculate kaolin as compared to water. The tri-valent citrate ion is still more efficient in this regard. That mineral acids are present in the soil has been demonstrated by many workers.

Na and K are soluble at the lower acidities. As the pH decreases, calcium and magnesium are leached from the soil solution. This loss results in a further decrease in the pH which brings into the soil solution iron and aluminum. From the data presented, it is evident that at the lower pH, iron and aluminum do not generally exist as the phosphates in which condition they are insoluble. Rather they are present as chlorides, nitrates, or sulfates.

The hardpan observed and previously reported upon indicated that the first cause in its formation was the deposition of SO_2 blown by the prevailing winds across the area from factories and railroads. The closer to the railroads and factories, the more prevalent and well defined was the hardpan. It is reasonable to suppose that the decreased pH was due to the formation of sulfuric acid.

With regard to the clay fraction, deflocculation prevails at the higher pH because of the high molarity of the soil solution in salts that can dissociate clay aggregates. Undissociated particles are constantly being washed down by gravitational water and are deposited at various depths, depending on the mechanical condition of the soil. Attending this phenomena is the fall in

pH, and the pH increases with the depth of the soil. Kelly (10) has shown that the lowest pH is generally at a depth of five or six inches from the surface. Thus the surface soil will lose sodium and potassium, and latterly, magnesium and calcium to the soil at greater depths. The result is that the clay particles are carried farther and farther down because of deflocculation.

When the acidity at the surface is sufficient to bring into the soil solution aluminum and iron, the formation of a hardpan layer begins. This pH is something less than 5.0, but may be slightly higher depending possibly on the phosphates present.

The iron and aluminum in dilute solutions are not adsorbed by the clay and so in the soil solution are carried down and with the decrease in acidity are deposited farther from the surface. The progressively decreasing pH results in more and more iron and aluminum being brought into the solution until sufficient quantities are present to cause the flocculation of the collected dispersed clay particles at some depth, depending on the pH.

The aggregated clay particles cause the formation of a tight layer very slowly permeable to water. When this layer has dried out, aluminum and iron are deposited around the aggregates as the oxides, mechanically binding the entire mass together and effectively preventing the passage of water.

The analyses of the soil, where the hardpan was found, for combined aluminum and iron show that there is the equivalent of approximately .0015 M $AlCl_3$ present. Figure 1 shows that considerably less than this concentration brings about the maximum flocculation of kaolin and also that at about this concentration aluminum is adsorbed. At the part of the same area of ground where no hardpan was found, the equivalent of less than 0.00004 M $AlCl_3$ was analyzed.

These figures may also be interpreted as indicating the toxic properties of soluble aluminum.

SUMMARY

Kaolin being used as criteria for all clays, determinations have been made on the degree of turbidity of a supernatant solution, on the final volume of the sedimented kaolin when treated with various solutions, on the conductivity of solutions for adsorption data, and on the sign and intensity of the charge on the particles. These experiments are preliminary and the data are only presented for their comparative value.

Several concentrations of HCl , $NaOH$, $NaCl$, Na_2SO_4 , $C_3H_5(COONa)_3$, $BaCl_2$, $CaCl_2$, $AlCl_3$, sucrose, and urea were used.

Different concentrations of the same electrolyte affect turbidities to a marked extent. Al clears the supernatant liquid in dilute solutions immediately after shaking, whereas the citrate and sulfate ions stabilize the suspension.

There is some correlation between turbidities and the final volumes of sediment. The most turbid solutions generally decrease the final volumes of

the kaolin to a marked extent, whereas nearly all the solutions of Al greatly increase the final volume.

Sucrose and urea have no effect on the kaolin whatever.

Of the ions used, Al and Na alone cause flocculation.

HCl is adsorbed at all dilutions to a great extent.

No other electrolyte used was adsorbed by the kaolin in the more dilute solutions.

A "negative adsorption" sequence is obtained: $\text{NaCl}, \text{NaOH} > \text{BaCl}_2 > \text{CaCl}_2 > \text{AlCl}_3$.

In concentrated solutions Al, HCl, and Ca and Ba reverse the sign of the charge on the particles most efficiently in the order given.

The degree of flocculation is apparently independent of the intensity of the charge on the particles.

Evidence is presented in support of the view that adsorption is not a function of pH in clays.

From the findings it seems that the amount of Al in a soluble form in the soil moisture depends on the amount of phosphate present in that soil as well as on the pH. That is, a great decrease in either may result only in a slight increase in the amount of Al present; a slight decrease in both will cause a great increase in the amount of Al and Fe in solution.

A theory is advanced to account for the formation of hardpan in acid clay soils.

REFERENCES

- (1) BLAIR, A. W., AND PRINCE, A. L. 1923 Studies on the toxic properties of soils. *Soil Sci.* 15: 109-129.
- (2) BOUYOUCOS, G. J. 1921 The concentration of the soil solution around the soil particles. *Soil Sci.* 11: 131-138.
- (3) BURGESS, P. S. 1923 Comparison of "Active" aluminum and hydrogen-ion concentrations of widely separated acid soils. *Soil Sci.* 15: 407-412.
- (4) CLARK, W. M. 1922 The Determination of Hydrogen Ions, ed 2, p. 41. Baltimore, Md.
- (5) DAYHUFF, W. C., AND HOAGLAND, D. R. 1924 The electrical charge on a clay colloid as influenced by hydrogen-ion concentration and by different salts. *Soil Sci.* 18: 401-408.
- (6) HALL, A. D. The Soil. New York.
- (7) HARKINS, M. D. 1925 The orientation of molecules in the surfaces of liquids. *Colloid Symposium Monog.* 2: 141-173.
- (8) HARTWELL, B. L., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soils on barley and rye. *Soil Sci.* 6: 259-277.
- (9) HILGARD, E. W. 1911 Soils. New York.
- (10) KELLY, A. P. 1923 Soil acidity, an ecological factor. *Soil Sci.* 16: 41-54.
- (11) LANGMUIR, I. 1916 The constitution and fundamental properties of solids and liquids. *Jour. Amer. Chem. Soc.* 38: 2221-2295.
- (12) LANGMUIR, I. 1916 The constitution of liquids with especial reference to surface tension phenomena. *Jour. Amer. Chem. Soc.* 38: 2221-2295.
- (13) LANGMUIR, I. 1916 *Chem. and Metall. Engin.* 15: 468-470.

- (14) LOEB, J. 1922 Proteins and the Theory of Colloidal Behavior. New York.
- (15) MAGISTAD, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181-225.
- (16) MATTSO, S. E. 1922 Die Beziehungen zwischen Ausflockung, Adsorption und Teilchenladung mit besonderer Berücksichtigung der Hydroxylionen. Berlin.
- (17) MATTSO, S. E. 1926 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 553-567.
- (18) MERRILL, G. P. 1906 Rocks, Rock Weathering and Soils, p. 14. New York.
- (19) MILLER, E. J. 1925 Adsorption by activated cane sugar. *Jour. Amer. Chem. Soc.* 47: 1270-1280.
- (20) MIRASOL, JOSE, J. 1920. Aluminum as a factor in soil acidity. *Soil Sci.* 10: 153-193.
- (21) NORTHROP, J. H. 1921-22. The stability of bacterial suspensions 1. A convenient cell for microscopic cataphoresis experiments. *Jour. Gen. Physiol.* 4: 629-6333.
- (22) SKEEN, J. R. 1925 A critical pH for the formation of hardpan in acid clay soils. *Soil Sci.* 20: 307-311.
- (23) SWANSON, C. O. 1923 Soil reaction in relation to calcium adsorption. *Jour. Agr. Res.* 26: 83-123.
- (24) VAN BEMMEL, J. M. 1879 Das Adsorptionsvermögen der Ackererde. *Landw. Vers. Sta.* 23: 265-393.
- (25) VAN BEMMEL, J. M. 1888 Die Adsorptionsvermögen und das Adsorptionsvermögen der Ackererde. *Landw. Vers. Sta.* 35: 69-121.
- (26) WARINGTON, R. 1900 Lectures on Some of the Physical Properties of Soil, p. 25, 28. Oxford.
- (27) WASHBURN, E. W. 1922 Physical chemistry and ceramics. *Jour. Franklin. Inst.* 193: 749-773.

DIFFERENCES IN THE HEAT OF REACTION BETWEEN ARTIFICIAL AND SOIL GELS OF SILICA, ALUMINA, AND IRON, WITH HYDROXIDES

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication June 1, 1926

While various studies on the heat of wetting of soils with water (1), were being conducted, the idea was suggested of investigating also the heat of reaction between soils and various chemical compounds. Accordingly, a series of studies was undertaken to ascertain whether there was any heat of reaction between various soils and different chemical compounds, and if so, measure the magnitude of this heat of reaction. Salts and hydroxides were employed. It was found that there was no measurable heat of reaction between the soils and salts, but that there was with the hydroxides, especially KOH and NaOH. A rather extensive study was then made of the heat of reaction of various soils with these hydroxides. In addition to the soils, soil colloids and artificial gels of silica, alumina, and iron were also used for purposes of comparison. The results obtained showed that whereas all the soils and soil colloids gave a considerable heat of reaction with the hydroxides, some of the artificial gels, especially silica, gave, in comparison, an enormous heat of reaction with the same hydroxides. For instance, the heat of reaction between silica gel and 3 *N* KOH was 146.7 calories per gram of material, whereas the highest value obtained in soils or colloids, excepting organic matter, was only 6.5 calories per gram. This is certainly an enormous difference, and raises many questions as to the similarity or dissimilarity in the physical and chemical characteristics of the gels of silica, alumina, and iron as they exist in the soil colloids and in the artificial state. It is the object of this paper, therefore, to present the data which show in detail the differences in the heat of reaction between soils, soil colloids, and artificial gels, with hydroxides.

PROCEDURE

The procedure followed in measuring the heat of reaction of materials in hydroxides, was essentially the same as that employed in determining the heat of wetting of soils with water, described in previous communications (1).

The total heat generated by the materials in the hydroxides included both the phenomenon of heat of wetting, and the heat of reaction. To obtain the latter, therefore, the heat of wetting with water was also determined and subtracted from the total for each respective material.

Four different hydroxides with various concentrations were tried, but the results of 3 *N* KOH and 3 *N* NaOH only will be given here.

EXPERIMENTAL DATA

In table 1 are presented the data on the heat of reaction of various types of soil, different soil colloids and artificial silica gel, alumina gel, and iron hydroxide.¹ For purposes of comparison, the heat of wetting of each material is also shown.

TABLE 1
Heat of wetting with water and heat of reaction with KOH and NaOH of soils and artificial gels

MATERIALS	HEAT OF WETTING WITH WATER	HEAT OF REACTION WITH KOH	HEAT OF REACTION WITH NaOH
	<i>calories per gram</i>	<i>calories per gram</i>	<i>calories per gram</i>
Freno fine sandy loam.....	1.12	0.17	
Amarillo fine sandy loam.....	4.97	0.43	
Rhode Island sandy loam.....	2.62	5.29	4.21
Ohio silt loam.....	2.109	1.15	1.12
Tennessee silt loam.....	2.45	2.48	1.62
Pennsylvania silt loam.....	2.81	1.58	1.48
Michigan silt loam.....	3.00	3.29	2.45
Michigan silt loam.....	1.55	1.91	1.81
Chester loam.....	2.39	1.11	0.95
Michigan Clay loam.....	4.98	3.72	2.52
Illinois Urbana Clay loam.....	4.00	3.92	2.18
California Yolo clay.....	6.52	3.52	1.94
Red clay—calcareous.....	2.81	2.01	1.12
Clay—calcareous.....	4.00	1.65	1.01
Ontonagon silt loam A ₁	3.94	5.09	3.76
Ontonagon silt loam A ₂	4.23	5.74	4.23
Ontonagon clay B ₁	6.50	4.26	2.28
Colloids from Ontonagon silt loam A ₂	7.51	5.89	
Colloids from Ontonagon Clay B ₁	9.12	4.62	
Colloids from Illinois Urbana clay loam.....	8.48	4.21	
Colloids from California Yolo clay.....	11.02	4.02	
Muck.....	29.50	25.82	20.14
Ferric hydroxide gel.....	9.390	13.65	
Alumina gel.....	21.95	61.23	
Silica gel.....	24.50	146.70	

The data in table 1 reveal many significant and interesting things. In the first place it is readily seen that all the materials employed gave a heat of reaction both with KOH and NaOH and that the magnitude of this heat of reaction varies tremendously with the different materials, especially between the soils and soil colloids, and the artificial gels. The magnitude of the heat of

¹ These three gels were kindly furnished by the Chemical Warfare Service.

reaction with KOH—in which the data are more complete—varies in the case of soils, from 0.17 calories in Fresno fine sandy loam, to 5.74 in Ontonagon silt loam A₂ horizon, to 28.50 in muck. In the case of soil colloids, it ranges from 4.02 calories in California Yolo clay to 5.89 in Ontonagon silt loam A₂ horizon. In the case of the artificial gels, it is 13.65 calories for ferric hydroxide gel, 61.23 for alumina gel, and 146.70 for silica gel. The differences between the figures for artificial gels and soils and even for the colloids, are certainly very great. The rather surprising thing is that the soil colloids gave no greater heat of reaction than the soils from which they were extracted. As will be seen the amounts are about the same in both cases.

The heat of reaction with both the KOH and NaOH does not vary in any constant manner in the different soils. Since muck gave a very great heat of reaction, it would logically seem that soils containing a high content of organic matter would give a correspondingly high value of heat of reaction. Such would seem to be the case, as in Rhode Island sandy loam and Urbana clay loam, but there are some cases in which the heat of reaction is comparatively high, but which contain hardly any organic matter. For instance, Ontonagon B₁ horizon contains practically no organic matter and yet it gives a greater heat of reaction than the majority of the soils investigated. The possible reason for these differences will be discussed.

NaOH gave a lesser heat of reaction than KOH with every material employed. The values are also irregular in the different soils.

A comparison next, of the heat of wetting with water with the heat of reaction with the hydroxides in the respective materials, shows that, with the exception of one case in the soils and one case in the soil colloids, the heat of wetting is greater than the heat of reaction with either KOH or NaOH, and that in many cases the differences in favor of the heat of wetting, are appreciable. For instance, the heat of wetting of Amarillo fine sandy loam is 4.97 calories, whereas its heat of reaction with KOH is only 0.43 calories. In Yolo clay, the heat of wetting is 6.52 calories, whereas its heat of reaction with KOH is 3.52 calories. In muck the heat of wetting is about the same as the heat of reaction with KOH, but much higher than that with NaOH. In the case of the artificial gels, however, the heat of reaction with KOH is far greater than the heat of wetting, especially in the case of the alumina and silica gels.

Again, a comparison of the heat of reaction between the soil colloids and the artificial gels, raise the question: Why should the heat of reaction in the soil colloids not be as great as that in the artificial gels, since, the chief constituents of the soil colloids consist of iron, alumina, and silica? The recent work of Robinson and Holmes (2), which contains the chemical analysis of a large number of colloids representing diverse types of soils throughout the United States, shows that the soil colloids are composed mainly of silica, alumina, and iron oxide, and that the silica may vary from 31.84 to 55.44 per cent, the alumina from 16.42 to 38.28 per cent, and the iron oxide from 4.66 to 16.67 per cent. If the silica, alumina and iron in the soil colloids were

in the same chemical and physical constitution, it would logically seem that they would react the same as in the artificial state. The fact that they do not react the same with the hydroxides, indicates that they are probably not of the same constitution. This heat of reaction could be caused by any one or by all of the following factors: (a) solution, (b) neutralization, (c) adsorption. The second and third factors could be of predominant importance, but in the present instance, they are negligible. It is the first factor that seems to be the main, if not the sole, cause of the heat of reaction manifested in the different materials with hydroxides. For instance, in the case of the artificial silica gel and the muck, which give the greatest amount of heat of reaction, it is actually seen that the materials have been dissolved and are in solution. This is especially true in the case of silica gel. It would seem, therefore, that the larger the amount of material dissolved, the greater will be the heat of reaction. The silica, alumina, and iron are apparently not of the same constitution in the soil colloids as in the artificial state, and cannot be dissolved as readily, and consequently their heat of reaction is less. Hence, this seems to be another evidence that the constitution, at least the physical constitution, of the soil colloids, is not the same as that of similar materials in the artificial state.

The differences in the heat of reaction obtained in the various soils could be explained, therefore, on the basis that different amounts of silica, alumina, iron, and organic matter are dissolved in the various soils.

It is of interest to note here that the heat of wetting gives values which are quite close to those of the artificial gels. For instance, in a former communication (1), it was shown that many soil colloids gave a heat of wetting as high as 16.192 calories (California adobe clay) as compared with 24.5 calories in silica gel. From the results with heat of wetting, it would seem that the silica, alumina, and iron gels in the soil colloids were very similar, but the results with the heat of reaction makes them appear radically different.

SUMMARY

When soils, soil colloids, and artificial gels of silica, alumina, and iron are treated with KOH and NaOH, heat is generated. This is the heat of reaction, which is in addition to that of heat of wetting.

The amount of heat of reaction in soils and soil colloids is rather small, whereas that in the artificial gels is comparatively tremendously high. In the mineral soils and soil colloids, it varies with KOH from 0.17 to 5.89 calories per gram of material; whereas in muck, it is 29.50 calories; in ferric hydroxide gel, 13.65; in alumina gel, 61.23; and in silica gel, 146.70. Apparently the difference in the heat of reaction between the artificial gels and the soil colloids is very great.

This heat of reaction seems to be due mainly to the dissolving effect of the hydroxides upon the materials. It is actually seen that all the artificial silica gel goes into solution.

Considering that the soil colloids are composed chiefly of the same gel constituents employed in this investigation; namely, silica, alumina, and iron, and yet they gave a very small amount of heat of reaction as compared with that of the artificial gels, it becomes evident that the silica, alumina, and iron in the soil colloids probably do not have the same constituents as in the artificial state. These results may be considered as additional evidence that the gels in the soil colloids are not be in the same condition as in the artificial state.

On the other hand, the heat of wetting values, as shown in previous works, does not show the soil colloids and artificial gels to be radically different.

REFERENCES

- (1) BOUYOUCOS, G. J. 1925 Heat of wetting as a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153-162.
- (2) ROBINSON, W. O., AND HOLMES, R. S. 1924 The chemical composition of soil colloids. U. S. Dept. Agr. Dept. Bul. 1311.

BOOK REVIEW¹

Plant Nutrition and Crop Production. By E. J. RUSSELL, University of California Press, Berkeley, 1926. ix + 115 pp., 37 fig., 21 pl.

This little volume consists of a series of lectures delivered in 1924 under the auspices of the Hitchcock foundation at the University of California, by the eminent scholar Sir John Russell, Director of the Rothamsted Experiment Station at Harpenden, England.

These lectures are a valuable addition to the efforts of the workers in scientific agriculture to elucidate the complex problems of plant environmental relationships in the light of our modern knowledge of chemistry and physiology. The title of the volume, "Plant Nutrition and Crop Production," may well be amplified by "a scientific inventory of the complex plant-soil system," for indeed it is a successful attempt to bring together the achievements of the scientific workers in the realm of agricultural sciences in establishing the fundamental relationships between plants and their environment.

A superficial examination of these lectures may mislead one into considering them a digest of the author's monograph, "Soil Conditions and Plant Growth." Although there is of necessity some repetitions, the subject under consideration is amplified with the most recent contributions of the rapidly growing knowledge of the physico-chemical and biological laws governing plant-soil reactions and behavior.

The first chapter—"The Study of Plant Nutrients"—deals primarily with the historical development of our knowledge of the "sources from which plants derive their substances." A skilful, short review of the earliest ideas on the subject ushers us in into the nineteenth century with an analysis of the contributions of de Saussure and Boussingault; these are followed by a critical review of the "turning point in the history of agricultural science," the researches of Liebig. Next come the classical experiments of the Rothamsted workers, the dawn of the fertilizer industry and practice and the principle involved. This leads the author to review briefly the fertilizer industry and the important fertilizer investigations in Europe and in the United States.

In the second chapter—"Positive Science and Exact Demonstration"—the author discusses the Mitscherlich logarithmic curve, which expresses the basic assumption that "the increased yield produced by increments of a nutrient is proportional to the decrement below the maximum producible if the nutrients were present in excess." He also presents some of the statistical data of Fisher from the Rothamsted Station "in an attempt to give quantitative

¹ This review was prepared by J. S. Joffe, of the New Jersey Agricultural Experiment Station.

expression to the facts of plant nutrition and growth." The timely subject, the rôle of farmyard manure in agriculture, is also interestingly discussed in the second chapter. The author seems to lean toward the view that organic matter cannot be replaced entirely by artificial manures. He is inclined to believe that the organics have a specific function outside of their value as improvers of tilth and moisture-holding capacity. This theory, which finds support in the experiments of Bottomley, is that organics possess a certain positive effect on plant growth which cannot be produced by artificial nutrients.

The third chapter—"Decay and the Living Plant"—is a discussion of the transformation of organic matter and of the nitrogen cycle. It takes up the rôle of specialized groups of microorganisms in the decomposition process of organic matter, the energy relationships and the carbon nitrogen ratio. The author attributes the direct microscopic method to Winogradsky, omitting the earlier work of Conn from the Geneva Station, United States, to whom Winogradsky gives due credit.

The last part of this chapter is a preamble to the fourth chapter—"The Soil Microorganisms; Can They be Controlled and Utilized?" This is a discussion of the methods used in controlling the various functions of the soil flora. The subject of soil inoculation, of course, is dealt with. A good share of this chapter is devoted to a discussion of the process, developed at the Rothamsted Station, of making manure from straw and other organic waste products by the addition of proper nutrients and by maintaining favorable conditions for the activities of microorganisms. The author did not discuss fully the subject of the soil population, but such omissions, as for instance autotrophy in soils, are probably a result of time limitations.

In the concluding chapter—"The Soil and the Living Plant"—Russell skims over the process of soil formation. He recognizes three divisions: first, the lateritic and silicate soils; second, the one that is determined by the presence or absence of calcium; third, that depending on the fate of the organic matter. A considerable discussion of soil colloids and base exchange is given.

The study of the soil complexes is one of the most fascinating problems for the future. The present investigations on basic exchange may reveal a good deal, but there are even greater hopes from the application of physical methods. It is possible that the use of X-rays might teach us as much about the structure of soils as in the hands of Sir William Bragg and his son they have taught about the structure of crystals.

There is also a brief discussion of soil moisture and gases in relation to plant growth.

A series of graphs, charts, and plates illustrate the points brought out in the subject matter.

A RESEARCH PROFESSORSHIP IN FOREST SOILS AT CORNELL UNIVERSITY

A gift of \$130,000 for the endowment of a research professorship in forest soils in Cornell University has been announced by President Livingston Farrand, who said that the Charles Lathrop Pack Forestry Trust, founded by Mr. Charles Lathrop Pack, President of the American Tree Association, in addition to endowing the chair, had made a further generous provision of funds for the operating expenses of the advanced line of investigation to be undertaken.

The work will be done in the New York State College of Agriculture, and the appointment of the professor will be announced shortly, President Farrand said. The chair will be named for Mr. Pack.

"In the northeastern hardwood area, extending over the Middle Atlantic States and as far west as Indiana and Illinois, the question of proper forest care and operation depends very largely upon a study of soil conditions, and practically no data on forest soils are at present available," President Farrand said. "The proposed line of investigation is a new development in forest research in this country. It will undertake to coördinate studies in several fields of science and apply what is learned to the special soil problems involved in the business of growing healthy forests.

"This research will necessarily deal with the chemistry and biology of soils. It will naturally have intimate relation with the field of heredity in tree growth, particularly as it should help to solve problems of adapting certain varieties of trees to given soils. And it will similarly have a bearing on the field of plant pathology, because of the relation that soil conditions bear to diseases of trees.

"Many of the timber grower's problems are complex. Their solution must be looked for in several fields, including these interrelated fields of soils, genetics, and forest pathology.

"This is the first time that such a comprehensive research on forest soils has been systematically undertaken in this country. The comprehensive study of forest soils is a new line of research everywhere, and the only specialized workers in it are a few scientists in Sweden, Russia, Finland, and Germany. Although the work to be done under Mr. Pack's endowment will deal directly with American forest problems, its results will have international interest and general scientific value."

ON THE EXISTENCE OF DAILY CHANGES IN THE BACTERIAL NUMBERS IN AMERICAN SOIL

H. G. THORNTON AND R. A. FISHER

Rothamsted Experimental Station

Received for publication May 6, 1926

The changing numbers of the various groups of microorganisms in a field soil must be a factor of vital importance to a proper understanding of the relationship of these organisms to their environment. It has long been known that the bacterial numbers are subject to seasonal changes. The numbers found at daily intervals has been studied recently by Cutler, Crump, and Sandon (2) at Rothamsted. These investigators found that in samples of soil taken at daily intervals from a plot on Barnfield, the bacterial counts obtained by the plate method showed striking variations from day to day. Before attributing these variations to true increases or decreases in the bacterial population counted, two possible sources of error must be considered.

In the first place there is a necessary experimental error in making the counts. In the work referred to, the platings were made by different workers, but in tests that were conducted, no variation in the technique could be detected, the factor limiting the accuracy of the counts being in nearly all cases the random distribution of the bacteria in the diluted suspension (4). This factor, which is calculable, was wholly insufficient to account for the fluctuations. The second source of error arises from the fact that samples taken at daily intervals are also taken at intervals in space, so that before the results are attributed to changes in bacterial numbers over a period of time, the possibility that they represent uneven distribution of the bacterial population in space must be excluded. This was done (2) by taking, on three occasions, 4 simultaneous samples from different parts of the plot. The counts obtained were in no case significantly different among the 4 samples. Furthermore, for 15 consecutive days, duplicate samples were taken 6 yards apart and the similar fluctuations in bacterial numbers were found in the duplicate series (2, p. 338). This rules out the possibility of accounting for the fluctuations found in Barnfield as being due to uneven distribution of the bacteria over the plot. Similar daily fluctuations in bacterial counts were found on Broadbalk (1) but there was no evidence as to whether the phenomenon was a general one occurring in other districts and under different soil conditions.

Recently, however, an important series of daily bacterial counts has been made at Washington, D.C., by Smith and Worden (6), who found fluctuations in the count from day to day. In this experiment, the plot selected was on a lawn, the sod of which was removed and the soil dug over.

The conditions were thus very different from those in the Rothamsted experiments. The method of sampling was also different, each count being made from a single core, instead of from a composite sample of 6 cores mixed together. Each day, however, the authors took 2 samples some distance apart and made separate counts from each, so that the influence on the results of the uneven distribution of the bacteria can be checked to some extent by comparing the variation in bacterial numbers between the 2 simultaneous samples with the difference found on consecutive days. The authors, on making such a comparison, concluded that the variation between simultaneous samples was greater than the fluctuations in the count from one day to the next. Their method of making this comparison is not valid, however, since they have compared the differences between individual simultaneous samples with day to day changes in the mean of 2 samples. Moreover, in sampling the plot, consecutive daily samples were taken from cores only 1 foot apart, whereas the 2 samples taken the same day were from spots several feet distant.

From the authors' data it is possible to make a decisive test of the reality of day to day changes in this particular set of counts, for if the whole of the variation observed in successive counts were due either to local differences or to the errors of random sampling, the two samples taken on the same day should show no positive correlation; indeed, since the simultaneous samples were taken from spots further apart than would be pairs chosen at random, a slight negative correlation should be anticipated. On the other hand, general changes in the bacterial numbers, affecting the whole plot, will tend to produce a positive correlation between simultaneous samples. If such a significant positive correlation between simultaneous samples exists, it excludes the possibility of the fluctuating bacterial counts being entirely due to uneven distribution of the bacteria. The authors' data are the more valuable because counts were made on 3 different media, namely, Löhnis' soil extract agar (5), Waksman's egg albumen agar (8), and Thornton's mineral salts agar (7). The counts on the first of these media from 32 pairs of samples taken between August 13 and September 19, show a positive correlation between simultaneous samples of $+0.4955$. The egg albumen agar was used for only 19 pairs of simultaneous samples taken between August 28 and September 19, which show a positive correlation of $+0.5673$. These two correlations must each be judged undoubtedly significant (3, p. 174). The mineral salts media was similar to that employed by Cutler, Crump, and Sandon for the Barnfield counts, though the temperature and period of incubation were different from those used at Rothamsted. The results obtained on this medium are thus more nearly comparable with the Barnfield counts. On it the 32 pairs of simultaneous counts obtained show a positive correlation of $+0.7744$. The probability against this occurring if the count differences were due to soil sample variation is about a million to one. Figures 1, 2, and 3 show the correlation between simultaneous samples on the three media. The existence of this correlation proves that the daily changes in the counts cannot be ascribed merely to uneven distribution of the bacteria within the area sampled.

The correlations stated above do not exclude the effect of slow changes in the bacterial numbers from the beginning to the end of the observations, changes to which Smith and Worden call attention, and which might perhaps properly be called seasonal. The authors have, therefore, recalculated the three values, eliminating a linear term representing the steady increase (or decrease) in the numbers (3, p. 171). The results show that the association between counts made on the same day is not to any large extent attributable to seasonal changes, but that there remain substantial and significant correlations when

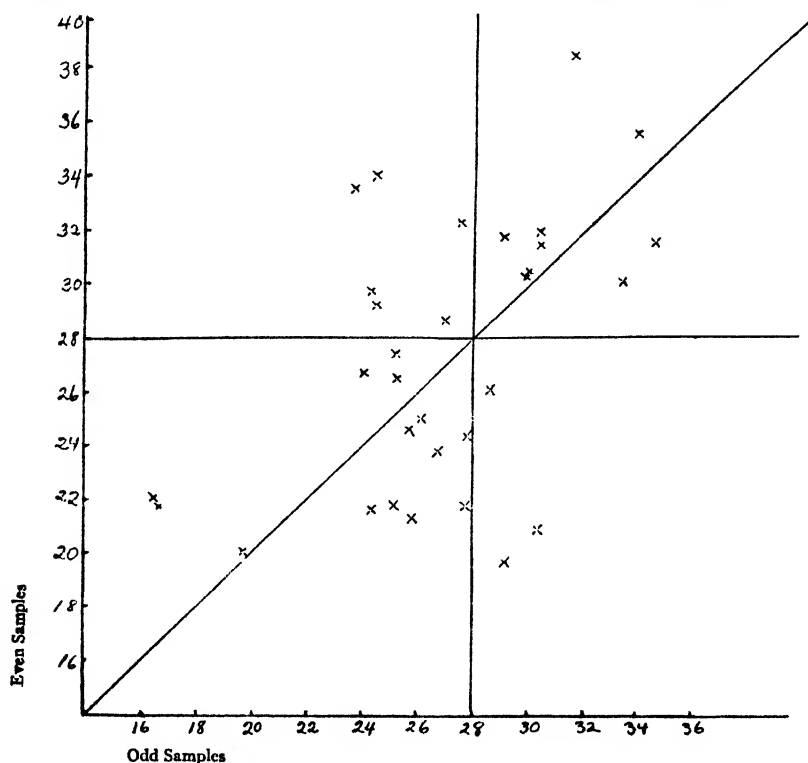


FIG. 1. CORRELATION BETWEEN SIMULTANEOUS SAMPLES PLATED ON SOIL EXTRACT AGAR (Smith and Worden's data)

the seasonal effect is eliminated. For the soil extract medium the correlation is reduced to $+0.3808$, a value which would occur by chance not more than once in 20 trials. For the egg albumen medium the correlation has risen to $+0.6615$ and for the mineral salts agar it has fallen to $+0.7060$, both these values being of quite unquestionable significance.

In speaking of the counts published by Smith and Worden as affording confirmation in American soil of continual changes similar to those observed at Rothamsted, it should be stated that we have never lost sight of the fact that systematic variations from day to day in the manipulative technique might

in many ways simulate actual variations in the bacterial population. In the case of the work at Rothamsted, the particular observations upon which our conclusions are based, as well as the whole body of comparable routine observations, have been so thoroughly tested and confirmed as to leave no residuum of doubt as to the objective reality of the phenomenon. Obviously, in the case of work done elsewhere, we have no such direct evidence of uniform technique and we can only demonstrate the *existence* of day to day variations of the same *type* as those observed at Rothamsted, without excluding the

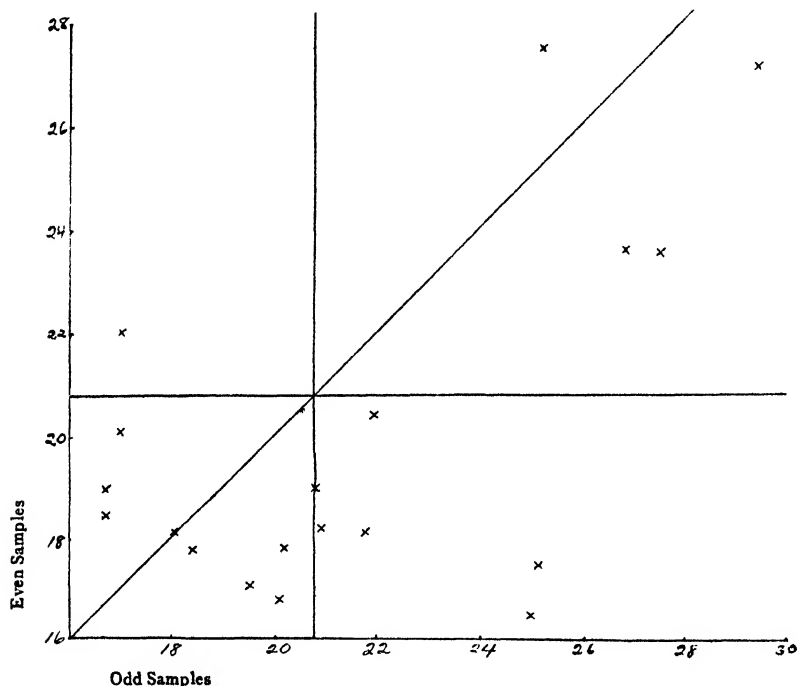


FIG. 2. CORRELATION BETWEEN SIMULTANEOUS SAMPLES PLATED ON EGG ALBUMEN AGAR (Smith and Worden's data)

possibility that variations in technique may have contributed to, or may be responsible for, the variations observed.

It is interesting that the closest correlation between counts from simultaneous samples was found when the mineral salts medium was used. If the counts obtained on this medium from individual cores be plotted separately (fig. 4) it is seen how similar are the changes in numbers in different regions of the plot. The fluctuations are more apparent on this medium than on either of the other media. The authors are probably right in attributing this to the selective action of the mineral salts medium. When using the plate method there is never any question of obtaining total bacterial counts, because no single

medium can possibly allow all physiological groups of the bacteria to develop. Consequently the comparative number of colonies that develop from a soil plating on a given medium is no criterion of the value of that medium for investigation. On the other hand it is essential to a satisfactory technique that

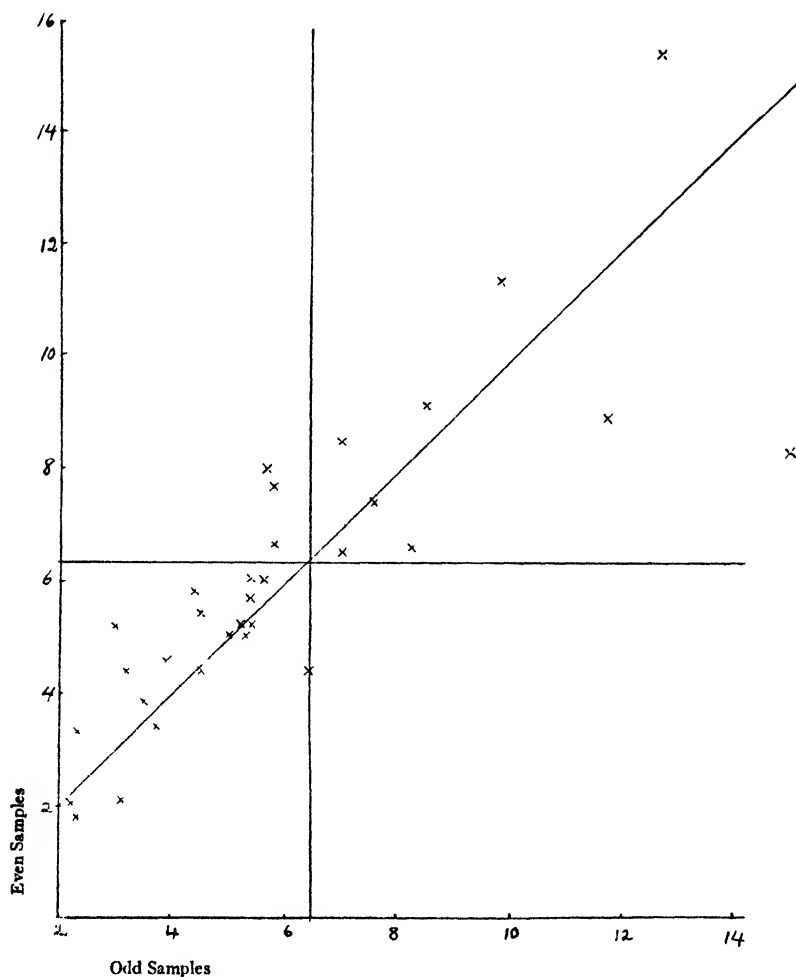


FIG. 3. CORRELATION BETWEEN SIMULTANEOUS SAMPLES PLATED ON MINERAL SALTS AGAR
(Smith and Worden's data)

the agreement between parallel plates should justify the assumptions upon which the dilution method is based, as can be tested by calculating χ^2 (4), and this agreement has so far been demonstrated only with the mineral salts agar. The greater number of colonies that appeared on the soil extract-medium probably indicates, as is suggested by Smith and Worden, that it is a

less selective medium than the mineral salts agar. For this reason it is less appropriate to the present type of investigation. It is unlikely that the numbers of all groups of bacteria fluctuate simultaneously. Some groups will increase while others will diminish in numbers. Consequently a selective medium is best suited to the study of these fluctuations because it diminishes the chance that a fall in the numbers of one group will be obscured on the plate counts by the increase of another group.

The work of Smith and Worden thus indicates that daily fluctuations in the state of the bacterial population which are similar in type to those already found at Rothamsted, exist in American soil, and also that the fluctuations occur under widely different soil conditions. There can be little doubt that the phenomenon is of general occurrence. It is possible, however, that only under exceptional conditions will the soil be sufficiently uniform to permit of its easy detection.

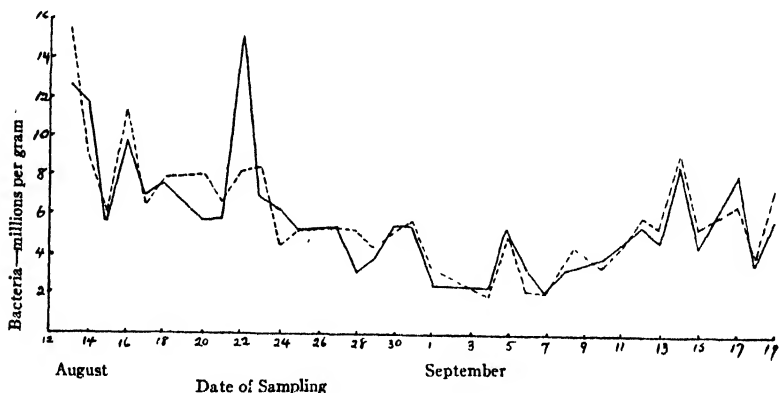


FIG. 4. DAILY COUNTS ON MINERAL SALTS MEDIUM

Bacterial numbers from the duplicate samples separately plotted. (Smith and Worden's data.)

SUMMARY AND ABSTRACT

The daily bacterial counts published by Smith and Worden show variations which cannot be explained by unequal distribution of bacteria in the soil, or by seasonal changes in bacterial numbers.

On all three media employed by them, significant positive correlations in bacterial numbers between simultaneous samples were obtained.

The similar daily fluctuations occurring in different parts of the plot show most clearly on Thornton's mineral salts medium.

Provided the manipulative technique of Smith and Worden was sufficiently uniform, the results afford evidence of the existence, in very different conditions, of fluctuations in bacterial numbers similar to those observed at Rothamsted.

REFERENCES

- (1) CUTLER, D. W., AND CRUMP, L. M. 1920 Daily periodicity in the numbers of active soil flagellates with a brief note on the relation of trophic amoebae and bacteria numbers. *Ann. Appl. Biol.* 7: 11-24.
- (2) CUTLER, D. W., CRUMP, L. M., AND SANDON, H. 1922 A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. *Phil. Trans. Royal Soc. (B)* 211: 317-350.
- (3) FISHER, R. A. 1925 Statistical Methods for Research Workers. Oliver and Boyd, Edinburgh.
- (4) FISHER, R. A., THORNTON, H. G., AND MACKENZIE, W. A. 1922 The accuracy of the plating method of estimating the density of bacterial population with particular reference to the use of Thornton's agar medium with soil samples. *Ann. Appl. Biol.* 9: 325-359.
- (5) LÖHNIS, F. 1913 Laboratory Methods in Agricultural Bacteriology. Philadelphia.
- (6) SMITH, N. R., AND WORDEN, S. 1925 Plate Counts of Soil Microorganisms. *Jour. Agr. Res.* 31. 501-517.
- (7) THORNTON, H. G. 1922 On the development of a standardized agar medium for counting soil bacteria with especial regard to the repression of spreading colonies. *Ann. Appl. Biol.* 9 241-247.
- (8) WAKSMAN, S. A. 1921 Microbiological analysis of soil as an index of soil fertility: II. Methods of the study of numbers of microorganisms in the soil. *Soil Sci.* 14: 283-298.

STUDIES OF THE REMOVAL OF NUTRIENTS FROM SUBSOIL BY ALFALFA

C. E. MILLAR¹

Michigan Agricultural Experiment Station

Received for publication June 4, 1926

Because of the deep rooting habit of alfalfa, frequent references have been made to the withdrawal of nutrients from the lower horizons of the soil profile by this crop. Insofar as the writer is aware, however, no data have been presented which prove that alfalfa actually does remove nutrients from this portion of the soil profile.

Alway, McDole, and Rost (1) found that inoculated alfalfa grew on certain subsoils of eastern Nebraska almost as luxuriantly as on the surface soils. On the other hand Harmer (2) found only two out of seven subsoils from Minnesota capable of producing as good a growth of inoculated alfalfa as the corresponding surface soils. It should be noted that the conditions of both these experiments were such as to allow the surface roots to absorb the nutrients.

In his excellent review of studies of the root habits of alfalfa, Weaver (6) points out that the nature of the root system is greatly modified by soil, climatic conditions, and height of water table. Garver (3) found that variety, frequency of cutting, and thickness of stand were potent factors in determining the nature of the root system. Weaver's own observations show that on fairly porous upland soils of not too restricted rainfall, the tap root is the main feature and that laterals below the first foot or eighteen inches are comparatively few and very short. Ten Eyck (5) on the other hand reports the major portion of the fibrous roots below the four-foot level.

The studies here reported were undertaken with the purpose of obtaining some definite information as to whether alfalfa, under the conditions of the experiment, actually does remove nutrients from the subsoil and further, whether it is capable of doing so when nutrients are added to the lower soil horizon.

METHOD OF PROCEDURE

The best method of procedure was considered to be one by which the roots could be restricted from ramifying in the surface soil without otherwise disturbing the plant. Glass cylinders about two inches in diameter and eight

¹ Professor in Soils.

inches long, which could be slipped over the crowns of the smaller plants, seemed to offer the greatest possibility, for the following reasons:

The amount of soil contained in the cylinder should offer minimum opportunity for nutrient absorption by the surface roots, especially since the water-retaining power of this small amount of soil would be so slight that it should be dried out very quickly after a rain. The glass walls of the cylinder would render impossible even the slightest trace of horizontal salt movement. The plants would thus be forced to rely on the nutrients which might be obtained through the lower portion of the root system, and the very small amount from the soil in the cylinders, or else draw on stored foods. The cylinder could be placed without any disturbance of the lower root systems.

In the spring of 1923 when growth was starting, the soil to a depth of about nine inches was carefully removed from around a number of two-year-old alfalfa plants growing in Coloma loamy sand. Cylinders were carefully slipped over these plants, so as not to damage the shoots, and placed with their tops just below the bottom of the crowns of the plants. The soil was replaced around the cylinders and the cylinders themselves were filled with soil from a depth of about three feet in some cases and in others with surface soil.

To determine whether nutrients added to the lower soil horizons would be absorbed and enhance plant growth, 500 cc. of Shive's three-salt solution of double strength was added at depths of 18 inches and 36 inches respectively. The solution was delivered very close to the tap root through an iron pipe inserted to the proper depth in an auger hole. The nutrient solution was first applied early in the spring about the time growth started and the application was repeated after the first and second cuttings. For comparison other groups of plants, not fitted with glass cylinders, were treated with the solution at the same depths. Starting with the application after the second cutting in 1924 the nutrient solution was made four times its usual strength, with the thought that possibly the quantities of salts in the double strength solution were not sufficient to induce a maximum response in the plants.

The growth from each plant was cut with shears, air-dried, and weighed individually, so that a complete record of the performance of each plant, cutting by cutting for a period of three years is available, with the exception of the second cutting in 1923, which was accidentally destroyed by a farm hand.

In 1924 examination showed that in some cases the roots had grown out around the bottom of the cylinders and had then turned upward and ramified in the surface soil (plate 1, fig. 1). This upward growth might be interpreted as indicating either a more plentiful supply of nutrients or water or possibly both in the surface soil than in the lower soil horizons. Roots which had so developed in the surface horizon were cut off.

DISCUSSION OF RESULTS OF PRELIMINARY EXPERIMENTS

Since these experiments were preliminary and included only a small number of plants under each treatment, it seems advisable to give only the general indications suggested by the results.

When the plants surrounded with cylinders containing surface soil are used as a basis of comparison and when each cutting is considered separately, the data indicate that during 1923 and 1924 nutrient solution applied at a depth of 18 inches stimulated quite markedly the growth of plants in cylinders filled with subsoil. By 1925, however, 50 per cent of the plants so treated had died and the growth of the others was inferior to that of plants in cylinders filled with subsoil but receiving no solution.

Strangely, plants not surrounded by cylinders but receiving solution at a depth of 18 inches did not show as much effect from the application, during the later cuttings in 1923 and 1924, as those surrounded by cylinders and receiving solution at the same depth. More of these plants lived through the season of 1925 but their growth was no more satisfactory than that of the plants in cylinders.

Addition of nutrient solution at a depth of 36 inches to plants in cylinders filled with subsoil resulted in an increased growth in 1923, but seemed to be of no special value to the crop in 1924. Because of the very poor condition of these plants no yields were taken in 1925.

Plants not in cylinders but receiving nutrient at a depth of 36 inches showed less influence from the treatment during 1923 than those in cylinders and receiving solution at the same depth. The second cutting of 1924 showed a gain, but for the other cuttings there was no advantage.

In general it may be said that inclosure in cylinders and the consequent exclusion of roots from the surface soil did not appear to be detrimental to alfalfa plants. On the other hand there was more tendency for plants so inclosed to respond to applications of nutrient salts in the lower soil horizons.

DISCUSSION OF RESULTS OF LATER EXPERIMENTS

In 1924 several treatments were selected as being best suited to answer the questions in mind, and groups of plants receiving these treatments were brought into the experiment. In addition a group of plants receiving nutrient solution in a trench about 3 or 4 inches deep around each plant was added to the series.

In compiling the data for the plants brought under treatment at this time, the yields from those plants not placed in cylinders and receiving no nutrient solution were taken as a basis for comparison. The data in table 1 give the percentage of each cutting as related to the corresponding cutting of the control plants. Since it was impossible to select plants of equal size for the various groups and since some plants winter killed, the first cutting each year is taken as a starting point or basis of comparison for the other cuttings.

The second cutting of the plants receiving solution at a depth of about 3 inches bore a lower relation to its check than the first cutting to its check. The third cutting shows a negligible increase over the percentage for the first cutting. It is evident, therefore, that the addition of the solution has not increased the yield for 1924. In 1925 the second and third cuttings show a

marked increase over the corresponding cuttings of the untreated plants, which indicates a distinct benefit from the added nutrient.

The plants not in cylinders but receiving solution at a depth of 36 inches show no influence of the treatment the first year. The second year, however, a distinct increase for the second and third cuttings is noted.

Plants not surrounded by cylinders showed no influence of nutrient solution applied at a depth of 60 inches, until the third cutting of 1925 when a distinct increase in growth was noted.

The first cutting of the plants in cylinders but receiving no solution was 36.3 per cent of the first cutting of the untreated plants, indicating that the plants in cylinders were much the smaller. The second cutting was virtually the same percentage but the third cutting showed a considerable increase. In 1925 the second and third cuttings showed an appreciable gain in percentage growth over the corresponding cuttings of the untreated plants. These data

TABLE 1
Yield of air-dry tops from alfalfa plants

The relation of each cutting to the corresponding cutting from the control plants

TREATMENT	1924			1925		
	First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Solution at 3 inches.....	111 7	96 5	113 9	75.9	101 2	117 2
Solution at 36 inches	89 1	81 3	86 7	50 9	66 7	77 0
Solution at 60 inches.....	51 2	47 8	49 2	31.1	30.1	57.2
Plants in cylinders; no solution . .	36 3	33 6	49 9	23 8	36.9	44.8
Plants in cylinders; solution at 36 inches	21 9	43 9	40 0	7 8	15 4	13.6
Plants in cylinders, solution at 60 inches	43 4	53 1	51 3	27.4	35.2	48 2

indicate that the cylinders were not limiting the growth of the plants and that an adequate supply of nutrients was being obtained. Whether the source of supply was the subsoil is an open question, since no withdrawal of stored material could be measured.

Plants in cylinders and receiving nutrient solution at a depth of 36 inches show a decided increase in percentage yield over the corresponding cuttings of the untreated plants, beginning with the first cutting each year. This indicates a decidedly beneficial effect of the solution, which agrees with the 1925 results from the plants receiving solution at the same depth but not inclosed in cylinders, but disagrees with the results for 1924.

Solution applied at a depth of 60 inches to plants in cylinders produced an appreciable increase in growth for the second and third cuttings for both years. This again does not agree with the results obtained when solution was applied at a similar depth to plants not in cylinders.

It is interesting to note that although the above data indicate no restriction of growth as a result of preventing root development in the surface soil by

means of glass cylinders, the plants so treated showed a decided increase in growth as a result of applications of nutrient solution at depths of 36 inches and 60 inches. Plants not inclosed in cylinders did not show such a definite response to additions of nutrients.

The data from both sets of experiments seem to justify the conclusion that nutrient solution applied at depths of 18 inches, 36 inches, and 60 inches increased the growth of alfalfa, especially when the roots were excluded from the surface soil. These results indicate that the lower portion of the root systems are capable of absorbing nutrients in appreciable quantities. Excavation of several specimens showed that a large development of fibrous roots had occurred at the depth of application of the nutrient solution. Plate 1, figure 2 shows this development in plants receiving nutrient at the 36-inch depth.

Nutrient solution applied around the surface roots of the plants did not stimulate growth the first year, and during the second year the increase was not so great as one might expect.

TABLE 2
Growth records of alfalfa plants
Weight of air-dry tops per cutting

PLANT NUMBER	1923		1924			1925		
	First cutting	Third cutting	First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting
	gm	gm	gm.	gm.	gm.	gm.	gm.	gm.
1	26 9	13 0	121 2	36 4	21 3	10 4	11 2	3 0
2	18 7	10 5	100 8	37 6	25 2	31 0	20.6	13 2
3	3 1	0 6	31.0	13 3	11 6	15 7	11.6	4 0
4	32.7	28 9	14 9	19 0	8 1	3 4
5	6 4	6 3	4 1	7 9	5 4	2.35
6	178 1	73 0	44 6	161 8	53 0	22 0

VARIATION IN GROWTH OF ALFALFA PLANTS

The erratic behavior of individual plants is one point emphasized by these studies. As an illustration, the performance of several plants by cuttings is given in table 2. It is evident that if definite conclusions are to be drawn, studies of alfalfa (and probably the same is true of other plants, having large facilities for food storage) should include large numbers of plants.

GROWTH OF ALFALFA PLANTS WITH ROOTS EXCLUDED FROM SURFACE 15 INCHES OF SOIL

In order to note the effect of yet more drastic treatment on the behavior of alfalfa, 2-inch glass cylinders 15 inches long were placed around seven plants. The cylinders were filled with quartz sand. The behavior of the plants under this extremely abnormal condition is shown in table 3.

It is interesting to note that notwithstanding the extremely adverse conditions under which these plants were placed, they all lived through the first year and until after the first cutting of the second year, when only one died. After the second cutting of 1924, another plant succumbed, all of the others living through the year. In the spring of 1925 only two plants were alive and these grew thriftily throughout the season. Whether the heavy mortality in the second year was due to a depletion of food reserves in the roots with a resulting decrease in size of root, increase in water content, and consequent susceptibility to winter injury as reported by Nelson (4) was not determined, but seems probable.

It is a remarkable fact that two plants should live and produce three cuttings annually for three years under the conditions of the experiment. It is evident

TABLE 3

Growth of alfalfa plants surrounded by glass cylinders 2 inches in diameter and 15 inches long
Weight of air-dry tops for each cutting

PLANT NUMBER	1923		1924			1925		
	First cutting	Third cutting	First cutting	Second cutting	Third cutting	First cutting	Second cutting	Third cutting
	gm.	gm	gm	gm.	gm	gm.	gm	gm
1	7 4	3 6	32 2	20.5	18 3
2	1 5	0 6	41 0
3	18 4	2 5	4 4	2 8
4	9 5	5 1	51 9	35 7	32 1	33 1	14 1	13 5
5	7 6	3 8	11 8	7 7	6 9
6	18 2	6 8	61 8	29 2	28 9
7	6 2	2 1	47 9	29 9	19 7	58 8	14 6	12 5

that nutrients must either have been absorbed from the lower soil horizons or else the plants were making use of stored reserves. From data which will appear in another paper, this latter suggestion seems very plausible.

ABSORPTION OF WATER BY THE LOWER ROOT SYSTEM

In working among the plants, the author noted that those which had been enclosed in cylinders the previous day showed no signs of wilting even during the hotter portions of the day. This seemed remarkable since the sand used for filling the cylinders contained only a small amount of moisture. To test still further their resistance to wilting, three plants were fitted with 8-inch cylinders and three with 15-inch cylinders filled with quartz sand. These operations were performed at 10:00 a.m. on May 4, which was a bright warm day. At 4:00 p.m. on the following day, which was also bright and warm, there were no signs of wilting. The conclusion is unavoidable that the lower roots were absorbing water at a sufficient rate to meet the transpiration requirement of the plants.

SUMMARY

The placing of glass cylinders 2 inches in diameter and 8 inches long around alfalfa plants, thus excluding the roots from the surface soil, did not appear to decrease the growth of the plants as measured by the weight of air-dry tops. Three cuttings were taken for two successive years.

Nutrient solution applied near the tap roots at various depths increased the growth of tops of plants inclosed in cylinders. Plants not having their root system restricted by cylinders showed little response to applications of nutrient solution.

When nutrient solution was applied to the lower soil horizons a considerable development of fibrous roots occurred in the region of application.

The plants under observation showed a great variation in behavior as measured by individual cuttings.

Five out of seven plants enclosed in glass cylinders 2 inches in diameter and 15 inches long filled with quartz sand, lived for two years, three cuttings being taken each year. Two of the plants lived for three years.

When plants with several inches of top growth were inclosed in 15-inch cylinders filled with dry quartz at 10:00 a.m. on a warm bright day, no evidence of wilting was apparent at 4:00 p.m. on the following day, which was also bright and warm.

REFERENCES

- (1) ALWAY, F. J., McDOLE, G. R., AND ROST, C. O. 1917 The Loess soils of the Nebraska, portion of the transition region: VI. The relative "rawness" of the subsoils. *Soil Sci.* 3: 9-35.
- (2) HARMER, P. M. 1918 The relative "rawness" of some humid subsoils. *Soil Sci.* 5: 393-401.
- (3) GARVER, S. 1922 Alfalfa root studies. U. S. Dept. Agr. Bul. 1087.
- (4) NELSON, N. T. 1925 The effects of frequent cutting on the production, root reserves, and behavior of alfalfa *Jour. Amer. Soc. Agron.* 17: 100-113.
- (5) TEN EYCK, A. M. 1905 The roots of plants. Kan. Agr. Exp. Sta. Bul. 127.
- (6) WEAVER, J. E. 1926 Root Development of Field Crops. New York.

PLATE 1

FIG. 1. These roots grew out around the bottom of the 8-inch cylinders and then turned upward and ramified in the surface soil.

FIG. 2. A large number of fibrous roots developed near the point of application of nutrient solution, at the 36-inch depth.



FIG. 1

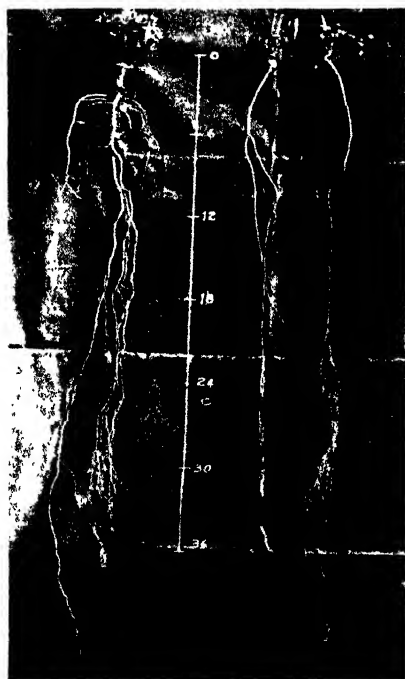


Fig. 2

THE MICROFLORA AND THE PRODUCTIVITY OF LEACHED AND NON-LEACHED ALKALI SOIL

J. E. GREAVES¹

Utah Agricultural Experiment Station

Received for publication July 2, 1926

The permanent reclamation of alkali soil rests upon the installation of underground drains and the leaching from the soil of the injurious constituents. The success of this method varies with the physical and chemical nature of the soil. With some soils it is a success whereas with others it is only a partial success or a total failure. Recent experiments show that these failures are due to the profound changes which the soluble salts have exerted on the composition of the soil; which in turn has changed so profoundly the structure of the soil that leaching becomes impossible. Such a condition can be overcome only by a preliminary treatment. Some soils, even after leaching, remain barren or produce only meagre crops for a number of years.

The non-productive period may be due to physical, chemical, or biological changes which have occurred within the soil because of its alkali content or the changes incidental to the leaching process. It is quite certain that the microflora would be profoundly influenced by the salt content of the soil. It is also reasonable to believe that considerable time would have to elapse after the leaching of such a soil before a normal active microflora would become established within it. The leaching of the soil would remove not only the injurious salts but considerable of the available plant-food. More plant-food would be rendered available only after the physical and chemical properties of the soil become suitable for bacterial growth and the soil became properly inoculated. Hence, this work was undertaken to determine the number of microorganisms, the biological changes occurring, and the productivity of natural and artificially leached and non-leached alkali soil which had been treated in various ways to re-establish its microflora.

PLAN OF EXPERIMENT

Two-gallon jars, provided with a half-inch hole near the lower edge were used. Into the holes were fitted rubber stoppers with glass tubes so that all drainage water could be collected, measured, and analyzed. A small quantity of glass wool was placed over the opening and the bottom of the jar was covered

¹ Valuable assistance in the analytical work was given by Professor Carter, Mr. Lund, Mr. Nelson, and Mr. Pulley.

to a depth of 2 inches with fine gravel. This was covered with a layer of glass wool. Sufficient soil to fill the pots was mixed with the requisite salts, packed into the pots, and the moisture content made to 20 per cent. Some pots contained untreated soil, others natural alkali soils, whereas still others contained synthetic alkali soil. The salt treatments were as follows:

12 pots were filled with the college farm soil untreated.

12 pots were filled with the college farm soil plus 2 per cent sodium chloride.

12 pots were filled with the college farm soil plus 2 per cent sodium sulfate.

12 pots were filled with the college farm soil plus 2 per cent sodium carbonate.

12 pots were filled with the college farm soil plus 1 per cent sodium chloride plus 1 per cent sodium sulfate.

12 pots were filled with the college farm soil plus 1 per cent sodium chloride plus 1 per cent sodium carbonate.

12 pots were filled with the college farm soil plus 1 per cent sodium sulfate plus 1 per cent sodium carbonate.

12 pots were filled with the college farm soil plus $2/3$ per cent each of sodium chloride, sodium sulfate, and sodium carbonate.

12 pots were filled with Corinne soil in which sodium chloride was the predominating salt.

12 pots were filled with Richland Acre soil in which sodium sulfate was the predominating salt.

12 pots were filled with Benson soil in which sodium carbonate was the predominating salt.

All of the soils stood for two months with a moisture content of 20 per cent before leaching so that the various reactions occurring because of the treatment would tend toward equilibrium. After this, six pots in each series were leached by keeping on them a "water head" as nearly constant as possible. All drain water was collected and analyzed. The results have been previously reported (6).

At the end of the leaching period (640 days) the soils were sampled and analyzed as to numbers, ammonifying, nitrifying, and nitrogen-fixing powers. After sampling, the pots were treated as follows:

Without further treatment two of the leached and two of the unleached pots from each series were seeded to crimson clover. Two of the leached and two of the unleached pots from each series received barnyard manure at the rate of fifteen tons per acre and were then seeded to crimson clover. Two of the leached and two of the unleached pots from each series received 50 cc. soil extract, which was made by shaking 100 gm. of a fertile soil in 1000 cc. of water, allowing it to settle, and then pipetting off 50 cc. The pots were then seeded to crimson clover.

* The plants including roots were harvested at the time of full bloom and total dry matter and percentage of nitrogen determined. The soil from each pot was then sampled and analyzed after which it was manured or inoculated with a water extract, as outlined above, and then seeded to barley. When ripe, the barley was harvested; the pots were resampled, retreated, and again seeded to barley. Hence, we have data on three samplings of the soil made at different times, and data on three crops—one of clover and two of barley. Four analyses were made on each sample of soil, hence, each result for numbers, ammonifying, nitrifying, and nitrogen-fixing powers represents the average of eight analyses made on two pots of soil receiving the same treatment.

COMPOSITION OF SOIL USED

The work was conducted on four different soils:

1. From the College Farm east of the Plant Industry Building.
2. From Corinne.
3. From Benson Ward.
4. From Richland Acres.

TABLE 1
Physical composition of soils

CONSTITUENTS	COLLEGE FARM SOIL	CORINNE SOIL	BENSON WARD SOIL	RICHLAND ACRES SOIL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sand	36 6	17.5	42.5	4.8
Fine sand	30 6	17.3	20.9	29.6
Coarse silt	18 8	28.5	19 0	27.0
Medium silt	6 0	15 9	3.8	13.1
Fine silt	2 3	5 3	2 3	10.2
Clay	3,5	7 4	6 1	9 4
Moisture, soluble salts, and loss ..	2 2	8 1	5 4	5 9

TABLE 2
Leaching necessary to remove the greater portion of the soluble salt from the soil

SOIL	TREATMENT	DURATION OF LEACHING PERIOD	WATER LEACHED
		<i>days</i>	<i>liters</i>
College	None	18	121
College	2 per cent NaCl	49	84
College	2 per cent Na ₂ CO ₃	274	140
College	2 per cent Na ₂ SO ₄	29	175
College	1 per cent NaCl plus 1 per cent Na ₂ SO ₄	25	153
College	1 per cent NaCl plus 1 per cent Na ₂ CO ₃	640	
College	1 per cent Na ₂ SO ₄ plus 1 per cent Na ₂ CO ₃	203	85
College	0.66 per cent each NaCl plus Na ₂ SO ₄ plus Na ₂ CO ₃	199	240
Corinne		211	79
Richland Acres		38	94
Benson Ward		139	247

Soil 1 was a good productive soil and was made unproductive by the addition of the salts listed above. Soils 2, 3, and 4 were natural alkali soils containing sufficient alkali to prevent the growth of all vegetation except a few salt-tolerant plants. The physical analyses of the four soils as determined by the Yoder (8) elutriator are given in table 1.

Soils 1 and 3 are sandy loams, whereas soils 2 and 4 are fine sandy loams. These soils should be more difficult to leach than a sand and much less difficult than a tight or heavy clay. However, they approximate the composition of

many of our alkali soils. Hence the results obtained in this work should give a lead to what may be expected in the field with the various alkali soils.

The soils were all leached until an analysis of the drain water indicated that the greater quantity of the soluble salts which could be removed by water had been washed from the soil. The time required and the quantity of water necessary to accomplish this, varied greatly with the different soils, as may be seen from the summarized results given in table 2.

The water passed rapidly through the normal soil during the first 48 hours yielding an average hourly leaching of 24.4 cc. After this there was a slight decrease, but this soil readily leached throughout the duration of the experiment. For a very short time the soil treated with 2 per cent sodium chloride leached readily, but it soon dropped to about one-third of the normal soil. The sodium-sulfate-treated soil leached more readily than did the untreated soil; whereas soil treated with sodium carbonate leached very slowly even at

TABLE 3
Recovery of salts from synthetic and natural alkali soils

SOIL	TREATMENT			NaCl RECOVERED		Na ₂ SO ₄ RECOVERED		Na ₂ CO ₃ RECOVERED	
	NaCl	Na ₂ SO ₄	Na ₂ CO ₃						
				gm	per cent	gm.	per cent	gm.	per cent
College	180			128 9	71.6				
		180				159 9	88 8		
			180					120.5	66.9
	90		90	85 0	94 5			22 0	24.5
	90	90		85 0	94 5	86.0	95 6		
Corinne.....		90	90			83 0	92 2	19.4	21.6
	60	60	60	54.8	91.3	58.6	97.7	26.0	43.3
				379 1		6.3			
				12.7		67.8			
Richland Acres . . .				97 0		40 6		103 7	
Benson Ward									

first, and by the end of one year only 1 cc. an hour was passing through the soil. This soil and drain water soon became dark in color. The soil puddled greatly and shrank in volume so that it did not fill the containers. Apparently the granular structure had been broken down completely. Although the leaching was continued in the case of some of the soil for nearly two years, considerable of the salt still remained in the soil, as may be seen from the summarized results given in table 3.

From 71.6 to 94.5 per cent of the chlorides, from 88.8 to 97.7 per cent of the sulfates, and only 21.6 to 66.9 per cent of the carbonates were recovered. Hence, in not a single case had all of the added salt been recovered although leaching had been as complete as was feasible without preliminary treatment.

NUMBER OF BACTERIA

The number of bacteria were determined by plating on modified synthetic agar having the following composition:

1000	cc. of distilled water.
10	gm. dextrose
0.5	gm. dipotassium phosphate (K_2HPO_4)
0.2	gm. magnesium sulfate ($MgSO_4$)
20	gm. powdered agar-agar

Before sampling, all large masses were broken up and the soil was thoroughly mixed by shaking in the original container. One-hundred gram portions of the soil were weighed on sterile paper into 200 cc. of sterile water. This was shaken for one minute and 1 cc. of this suspension was transferred to 99 cc. of sterile water. The dilution was continued in 9 cc. of sterile water and plates were made so as to give dilutions of 1 to 20,000 and 1 to 200,000. They were incubated at 28°C. for seven days and then counted. Three sets of plates were made from each soil and the results averaged. Hence, each reported result represents six determinations made on the duplicate pots receiving the same treatment. The average results for the chloride-treated soil are given in table 4.

The determinations made in 1921 were on composite samples of the soil. The six pots in each series which had received the same treatment were sampled and composited.

The leaching of the normal soil increased the bacterial content 24 per cent. This could not have come from the organisms carried in by the leach water for two reasons: first, the same water was used in irrigating the unleached soil, hence, its effect would have been the same on the microflora of both leached and unleached soils, second, it could not have been due to the soil retaining the microorganisms of the water used in leaching, as ordinary tap water, which is piped from a mountain spring, was used and repeated counts of the water during the leaching never showed a bacterial content of over 30 to each cubic centimeter. The increase could be due to the water breaking up the clumps of bacteria within the soil, under which condition the increase would be only apparent; or it might be due to a chemical or physical action upon the soil or its constituents which renders it more suitable to bacterial growth. The water may have removed chemical or biological substances which are inimical to bacterial growth. That this entity is a bacteriophage is a feasible explanation.

The 2 per cent sodium chloride at the first sampling had reduced the bacterial numbers to 54 per cent normal, but after this soil was leached, they increased 50 per cent. This is 26 per cent above the normal leached soil. The combination of sodium chloride and sodium sulfate slightly increased the numbers. Leaching of this soil increased the numbers to about the same extent as leaching of the sodium-chloride-treated soil. The combination of sodium

chloride and sodium carbonate is more toxic than sodium chloride and sodium sulfate or sodium chloride alone. This soil when leached contained seven times as many bacteria as the normal soil. The combination of the three salts

TABLE 4
Colonies developing upon synthetic agar from soil with and without chlorides

TREATMENT	COLONIES DEVELOPING IN 7 DAYS		
	1921	July, 1922	August, 1923
	thousands	thousands	thousands
Untreated soil	2,573	3,300	2,250
Untreated soil leached	3,182	3,500	3,150
Untreated soil leached + 15 T. manure		3,150	5,150
Untreated soil leached + soil extract		3,100	6,100
Soil + 2 per cent NaCl	1,387	1,850	3,250
Soil + 2 per cent NaCl leached	3,870	2,700	4,650
Soil + 2 per cent NaCl + 15 T. manure		1,500	3,900
Soil + 2 per cent NaCl leached + 15 T. manure		4,550	4,450
Soil + 2 per cent NaCl + soil extract		600	2,450
Soil + 2 per cent NaCl leached + soil extract		5,650	6,350
Soil + 1 per cent each NaCl and Na ₂ SO ₄	2,752	3,750	1,450
Soil + 1 per cent each NaCl and Na ₂ SO ₄ leached	3,750	4,000	4,300
Soil + 1 per cent each NaCl and Na ₂ SO ₄ leached + 15 T. manure		5,200	4,500
Soil + 1 per cent each NaCl and Na ₂ SO ₄ leached + soil extract		4,150	1,850
Soil + 1 per cent each NaCl and Na ₂ CO ₃	1,657	8,450	4,650
Soil + 1 per cent each NaCl and Na ₂ CO ₃ leached	18,750	15,050	4,350
Soil + 1 per cent each NaCl and Na ₂ CO ₃ leached + 15 T. manure		10,600	7,050
Soil + 1 per cent each NaCl and Na ₂ CO ₃ leached + soil extract		12,850	3,650
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	3,042	10,250	2,600
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ , leached	7,200	9,450	2,650
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + 15 T. manure		5,400	3,000
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + soil extract		9,200	2,100
Corinne alkali soil	220	600	1,800
Corinne alkali soil + 15 T. manure		300	1,250
Corinne alkali soil leached	885	3,150	5,450
Corinne alkali soil leached + 15 T. manure		2,700	1,950
Corinne alkali soil leached + soil extract		7,050	3,550

stimulated, and when this was washed out the stimulation became even greater. The Corinne alkali soil contained fewer organisms than any of the synthetic alkali soils; there was only a quarter of a million whereas two years later there was nearly two millions—leaching increased the number 400 per cent.

The evidence is strong that this soil contains something which is limiting the number of bacteria within it, and that this limiting factor is removed by leaching or by small quantities of the alkali. The factor cannot be protozoa as they would not be washed from the soil as readily as the bacteria; it could be a filterable virus or a toxic product. It would have to be assumed that the virus is more readily washed from the soil than are the bacteria and that it is more sensitive to alkalies. It is readily conceivable that the limiting factor could be a bacterial poison which is removed by leaching, but it would have to be assumed that the various salts neutralize its toxicity. The bacterial numbers in the second sampling are usually higher than in the first or third. By using the normal unleached soil in each case as a basis, leaching may be seen to increase the bacterial numbers 20 per cent; leaching and manure, 140 per cent; and leaching the soil and soil extract, 67 per cent. Two per cent sodium chloride reduces the bacterial content to 92 per cent of normal, but leaching increases it to 30 per cent above normal. The manure, when applied to the soil containing sodium chloride, has little influence on numbers; but when applied to sodium-chloride-leached soil, increase the numbers 30 per cent over that receiving no manure. Soil extract applied to the alkali soil apparently retards, but applied to the leached soil, increases the numbers 41 per cent over the manure-treated soil. Similar relationships exist in the combinations of the salts, with the exception of the sodium carbonate. Here the manure and soil extract fail to increase the numbers.

Although large variations occur in the numbers, it is safe to conclude that with this soil, leaching increases the bacterial content. Two per cent sodium chloride greatly decreases the numbers, but a combination of the chloride with the sulfate or carbonate or with both increases the numbers. Judged by the number of bacteria developing on synthetic media, it would appear from these results that the extract from the fertile soil is just as effective in increasing the numbers of microorganisms as is barnyard manure.

The results for the sulfates are given in table 5. Judged from the number of microorganisms developing on synthetic media, sodium sulfate is not nearly so toxic as is sodium chloride, a conclusion which corresponds with the results obtained with the higher plants. The combination of sodium sulfate and sodium carbonate stimulates to an even greater extent than does the sodium-chloride-sodium-carbonate combination. Although the increase due to leaching is not so pronounced as it was in the sodium chloride treated soil, it unmistakably manifests itself.

The manure and soil extract when applied to the soil usually increase the bacterial numbers. In this regard the soil extract is again just as effective as is the organic manure.

The results for the carbonate are given in table 6.

Sodium carbonate is much more toxic than either of the other salts. Two per cent sodium chloride reduced the bacterial numbers 11 per cent and leaching increased them 38 per cent, as an average compared with the normal soil.

The average results for the sodium sulfate as compared with the normal soil are within experimental error. But the sodium carbonate treated soil shows a decrease of 24 per cent due to the carbonate and an increase of over 830 per

TABLE 5
Colonies developing upon synthetic agar from soil with and without sulfates

TREATMENT	COLONIES DEVELOPING IN 7 DAYS		
	1921	July, 1922	August, 1923
	thousands	thousands	thousands
Untreated soil	2,573	3,300	2,250
Untreated soil leached	3,182	3,500	3,150
Untreated soil leached + 15 T. manure		3,150	5,150
Untreated soil leached + soil extract		3,100	6,100
Soil + 2 per cent Na_2SO_4	3,605	2,350	2,200
Soil + 2 per cent Na_2SO_4 leached	3,970	2,450	2,250
Soil + 2 per cent Na_2SO_4 + 15 T. manure		3,300	1,750
Soil + 2 per cent Na_2SO_4 leached + 15 T. manure		3,500	3,200
Soil + 2 per cent Na_2SO_4 + soil extract		3,800	2,300
Soil + 2 per cent Na_2SO_4 leached + soil extract		3,800	2,200
Soil + 1 per cent each Na_2SO_4 and NaCl	2,752	3,750	1,450
Soil + 1 per cent each Na_2SO_4 and NaCl leached	3,700	4,000	4,300
Soil + 1 per cent each Na_2SO_4 and NaCl leached + 15 T. manure		5,200	4,500
Soil + 1 per cent each Na_2SO_4 and NaCl leached + soil extract		4,150	4,850
Soil + 1 per cent each Na_2SO_4 and Na_2CO_3	1,745	16,650	1,300
Soil + 1 per cent each Na_2SO_4 and Na_2CO_3 leached	8,100	13,800	1,800
Soil + 1 per cent each Na_2SO_4 and Na_2CO_3 leached + 15 T. manure		19,500	2,200
Soil + 1 per cent each Na_2SO_4 + Na_2CO_3 leached + soil extract		7,700	1,850
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3	3,042	10,250	2,600
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached	7,200	9,450	2,650
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + 15 T. manure		5,400	3,000
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + soil extract		9,200	2,100
Richland acre alkali soil	1,452	6,000	3,550
Richland acre alkali soil + 15 T. manure		14,550	2,200
Richland acre alkali soil leached	1,722	5,050	3,000
Richland acre alkali soil leached + 15 T. manure		2,600	5,000
Richland acre alkali soil leached + soil extract		3,500	2,000

cent due to the leaching. Hence, the numbers increase enormously after the salt is removed. This is surprising, for the soil after leaching is in very poor physical condition. Moreover, much of the organic matter and large quantities of the nitrogen have been removed. The readily available phosphorus

and potassium had been rendered soluble and leached from the soil (6). Hence, it would appear that both physical and chemical conditions have been left less favorable for bacterial multiplication, yet the bacterial numbers greatly

TABLE 6
Colonies developing upon synthetic agar from soil with and without carbonates

TREATMENT	COLONIES DEVELOPING IN 7 DAYS		
	1921	July, 1922	August, 1923
	thousands	thousands	thousands
Untreated soil	2,573	3,360	2,250
Soil leached	3,182	3,500	3,150
Soil leached + 15 T. manure		3,150	5,150
Soil leached + soil extract		3,100	6,100
Soil + 2 per cent Na_2CO_3	982	3,750	1,450
Soil + 2 per cent Na_2CO_3 leached	11,000	10,650	3,550
Soil + 2 per cent Na_2CO_3 leached + 15 T. manure		18,250	2,600
Soil + 2 per cent Na_2CO_3 + 15 T. manure		5,100	2,050
Soil + 2 per cent Na_2CO_3 + soil extract		5,050	800
Soil + 2 per cent Na_2CO_3 leached + soil extract		15,000	4,300
Soil + 1 per cent each Na_2CO_3 , NaCl	1,657	8,450	4,650
Soil + 1 per cent each Na_2CO_3 , NaCl leached	18,750	15,050	4,350
Soil + 1 per cent each Na_2CO_3 , NaCl leached + 15 T. manure		10,600	7,050
Soil + 1 per cent each Na_2CO_3 , NaCl leached + soil extract		12,850	3,650
Soil + 1 per cent each Na_2CO_3 , and Na_2SO_4	1,745	16,650	1,300
Soil + 1 per cent each Na_2CO_3 , and Na_2SO_4 leached	8,100	13,800	1,800
Soil + 1 per cent each Na_2CO_3 , and Na_2SO_4 leached + 15 T. manure		19,500	2,200
Soil + 1 per cent each Na_2CO_3 , and Na_2SO_4 leached + soil extract		7,700	1,850
Soil + 0.66 per cent each Na_2CO_3 , NaCl , Na_2SO_4	3,042	10,250	2,600
Soil + 0.66 per cent each Na_2CO_3 , NaCl , Na_2SO_4 leached	7,200	9,450	2,650
Soil + 0.66 per cent each Na_2CO_3 , NaCl , Na_2SO_4 leached + 15 T. manure		5,400	3,000
Soil + 0.66 per cent each Na_2CO_3 , NaCl , Na_2SO_4 leached + soil extract		9,200	2,100
Benson alkali soil	40	610	150
Benson alkali soil + 15 T. manure		645	1,550
Benson alkali soil leached	267	1,755	2,100
Benson alkali soil leached + 15 T. manure		1,020	3,750
Benson alkali soil + soil extract		840	550
Benson alkali soil leached + soil extract		1570	2,500

increase. The most plausible explanation seems to be that there is within the soil some biological factor that is rendered inactive by the alkali, and when part of the alkali is removed by leaching, the bacteria multiply unhindered.

Moreover, all of the salt treatments give a great reduction at first, but after a time the number of organisms in the alkali treated soil becomes more numerous. This raises the very important question: Do the normal species develop into alkali resistant strains or is a different microflora developing within the soil? This must be answered by future work.

AMMONIFICATION

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering them with petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to 18 per cent with sterile distilled water. The samples were incubated at 28° to 30°C. for 4 days and the ammonia was determined by transferring them to Kjeldahl flasks with 250 cc. of distilled water, adding 2 gm. of magnesium oxide, and distilling into 0.1*N* sulfuric acid. The determinations were all made in triplicate and compared with sterile blanks. Hence, each reported result represents the average of six determinations made on the duplicate pots. The average results for the chloride-treated soil, stated as milligrams of ammonia, recovered from 100 gm. of soil are given in table 7.

The leaching of the normal soil increased its ammonifying powers 12 per cent. The stimulation is greatest at the time of the first sampling and had not fully disappeared at the end of two years. The manure produced no measurable effect the first year, but the second year there was a gain in ammonia. The soil extract had no effect on the non-alkali soil.

Two per cent sodium chloride reduced ammonification to 10 per cent of normal. The addition of manure or soil extract to the unleached soil had no influence. Leaching the soil brought it back to 85 per cent of normal, and the addition of manure to the leached soil restored it to its normal ammonifying efficiency. The soil extract was not as effective in this regard as was the manure.

A mixture of sodium chloride and sodium sulfate is less toxic, measured in terms of ammonification, than is sodium chloride alone. Moreover, the leaching restores such a soil to its original ammonifying powers. The addition of soil extract or manure raises its ammonifying powers above the normal. Here also the manure is more efficient than is the soil extract.

The mixture of sodium chloride and sodium carbonate is not as toxic as is the single salt; moreover, such a soil is more easily restored to its normal ammonifying powers. In restoring ammonifying efficiency soil extract and manure are about equally efficient.

A mixture of the three salts is slightly more toxic than the two. Neither leaching, soil extract, nor manure, when used singly or in combination, fully restored the ammonifying powers of the soil.

The Corinne alkali soil produced very little ammonia. The manure and soil extract were both without effect on the unleached soil. The leaching of the soil increased its ammonifying powers, which were still less than one-half

those of the non-alkali soil. At the end of two years neither manure nor soil extract had brought this soil up to a normal ammonifying efficiency. But the leached soil in the absence of treatment did not greatly increase in ammo-

TABLE 7
Ammonia found in soil with and without sodium chloride after 4 days' incubation

TREATMENT	AMOUNT OF AMMONIA FOUND		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil	89	91	66
Soil leached	107	96	74
Soil leached + 15 T. manure		97	103
Soil leached + soil extract		90	81
Soil + 2 per cent NaCl	10	8	9
Soil + 2 per cent NaCl leached	65	59	84
Soil + 2 per cent NaCl + 15 T. manure		9	9
Soil + 2 per cent NaCl leached + 15 T. manure		73	114
Soil + 2 per cent NaCl + soil extract		9	13
Soil + 2 per cent NaCl leached + soil extract		66	69
Soil + 1 per cent each NaCl, Na ₂ SO ₄	15	8	31
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	94	106	74
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + 15 T. manure		150	102
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + soil extract		112	83
Soil + 1 per cent each NaCl, Na ₂ CO ₃	45	22	32
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached	70	49	118
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + 15 T. manure		70	113
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + soil extract		73	118
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	19	10	34
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached	64	67	73
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + 15 T. manure		58	103
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + soil extract		62	70
Corinne alkali soil	2	2	5
Corinne alkali soil + 15 T. manure		1	6
Corinne alkali soil leached	40	16	57
Corinne alkali soil leached + 15 T. manure		32	89
Corinne alkali soil + soil extract		1	7
Corinne alkali soil leached + soil extract		12	96

nifying efficiency during the two years. In restoring the ammonifying powers of the soil, the soil extract was just as efficient as was the manure. The results indicate that one of the greatest benefits derived from the manure was the reseeded of the soil with an appropriate microflora.

The results for the sulfates are given in table 8.

From the viewpoint of ammonification, sulfates evidently are not as toxic as are chlorides. Such a soil is more easily reclaimed, the mere leaching of the

TABLE 8
Ammonia found in soil with and without sodium sulfate after 4 days' incubation

TREATMENT	AMOUNT OF AMMONIA FOUND		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil	89	91	66
Soil leached	107	96	74
Soil leached + 15 T. manure		97	103
Soil leached + soil extract		90	81
Soil + 2 per cent Na_2SO_4	22	43	63
Soil + 2 per cent Na_2SO_4 leached	93	93	80
Soil + 2 per cent Na_2SO_4 + 15 T. manure		47	73
Soil + 2 per cent Na_2SO_4 leached + 15 T. manure		118	113
Soil + 2 per cent Na_2SO_4 + soil extract		36	45
Soil + 2 per cent Na_2SO_4 leached + soil extract		105	38
Soil + 1 per cent each Na_2SO_4 , NaCl	15	8	31
Soil + 1 per cent each Na_2SO_4 , NaCl leached	94	106	74
Soil + 1 per cent each Na_2SO_4 , NaCl leached + 15 T. manure		150	102
Soil + 1 per cent each Na_2SO_4 , NaCl leached + soil extract		112	83
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3	51	45	69
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached	103	63	109
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + 15 T. manure		69	130
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + soil extract		58	103
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3	19	9	34
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached	64	67	73
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + 15 T. manure		58	103
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + soil extract		62	70
Richland acre alkali soil	11	11	50
Richland acre alkali soil + 15 T. manure		38	70
Richland acre alkali soil leached	24	29	66
Richland acre alkali soil leached + 15 T. manure		32	86
Richland acre alkali soil + soil extract		10	57
Richland acre alkali soil leached + soil extract		24	42

soil being sufficient to restore it to normal. Both the soil extract and the manure bring the leached soil to above normal. Moreover, the addition of the manure to the unleached soil is sufficient in some cases to restore the soil

to its normal ammonifying powers. This of course, is only temporary, and as the organic matter decays, the toxic properties of the salts reappear.

TABLE 9

Ammonia found in soil with and without sodium carbonate after 4 days' incubation

TREATMENT	AMOUNT OF AMMONIA		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil.....	89 0	91.0	66.0
Soil leached.....	107.0	96.0	74.0
Soil leached + 15 T. manure.....		97.0	103.0
Soil leached + soil extract.....		90 0	81.0
Soil + 2 per cent Na_2CO_3	22.4	46 4	32.8
Soil + 2 per cent Na_2CO_3 leached.....	92' 8	93.0	77.5
Soil + 2 per cent Na_2CO_3 + 15 T. manure.....		46.8	73.4
Soil + 2 per cent Na_2CO_3 leached + 15 T. manure.....		118 9	112.5
Soil + 2 per cent Na_2CO_3 + soil extract.....		35.9	42.7
Soil + 2 per cent Na_2CO_3 leached + soil extract.....		100 2	115 0
Soil + 1 per cent each Na_2CO_3 , NaCl	15.0	8.0	31.0
Soil + 1 per cent each Na_2CO_3 , NaCl leached.....	94.0	106 0	74 0
Soil + 1 per cent each Na_2CO_3 , NaCl leached + 15 T. manure.....		150.0	102 0
Soil + 1 per cent each Na_2CO_3 , NaCl leached + soil extract.....		112.0	83.0
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3	51.0	45.0	69 0
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached.....	103.0	63.0	109.0
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + 15 T. manure.....		69.0	130.0
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + soil extract.....		58.0	103.0
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3	19.0	9.0	34.0
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached.....	64.0	67 0	73.0
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + 15 T. manure.....		58.0	103 0
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + soil extract.....		62.0	70.0
Benson alkali soil.....	8.8	10.7	12.7
Benson alkali soil + 15 T. manure.....		24.0	16.5
Benson alkali soil leached.....	46.5	52.5	93.4
Benson alkali soil leached + 15 T. manure.....		69 7	122.7
Benson alkali soil + soil extract.....		8.2	12.4
Benson alkali soil leached + soil extract.....		52.2	107.1

The results are significant in that they are in keeping with those obtained when the plant is taken as the index. Moreover, it is the common experience that the sulfate alkali soil is more readily reclaimed than is the chloride. The natural alkali soil has a lower ammonifying power than has the artificially

produced, and is harder to reclaim. The artificial soil of this series is restored by leaching, but it requires both leaching and re-inoculation to restore the natural soil.

The results for the carbonates are given in table 9.

Sodium carbonate is more toxic to ammonifiers than is sodium sulfate, but is less toxic than is sodium chloride—2 per cent sodium chloride reduced ammonification to 10 per cent of normal, sodium carbonate to 41 per cent of normal, and sodium sulfate to 52 per cent of normal. Moreover, it is more easily returned to its normal ammonifying power than is the sodium chloride carrying soil. Both manure and soil extract are efficient in reseeded the leached soil. Moreover, it is interesting to note that the leached manure-treated soils shows a high ammonifying power, the alkali having a stimulating influence. All of these results are in direct accord with the results which were obtained by mixing the salt directly with the soil and then incubating (3).

NITRIFICATION

The nitrifying power of the soil was determined similarly to the ammonifying power, except that the samples in the former case were incubated for 21 days. The moisture content was made up weekly to the initial percentage and the nitric nitrogen determined as outlined elsewhere (5). The average results for the chloride-treated soil are given in table 10.

The leaching of the soil increased nitrification 8 per cent. Hence, we find that the leaching of this fertile soil has increased the numbers, ammonification, and nitrification, thus showing that the water must remove some limiting factor in the bacterial growth in this soil. The addition of manure increases, whereas the addition of the soil extract decreases, nitrification.

Two per cent of sodium chloride reduces nitrification to 12 per cent of normal. Both manure and soil extract are valueless when judged by nitrification. However, the soil extract is more valuable than is the manure in restoring nitrification in the leached soil.

As it is only after the soil has been leached and re-inoculated that nitrification is normal, these results would point to the conclusion that 2 per cent sodium chloride in a soil for some time not only inactivates the nitrifying organisms but actually destroys them. A combination of the two salts is just as toxic as is the single salt. The combination of the three salts is toxic at first but later decreases. Whether this is due to a chemical combination of the salts with soil constituents or whether the organisms become accustomed to the alkali salts cannot be decided from these results. They point to the conclusion that alkali soil after leaching must be reseeded with the nitrifying organisms before high nitrification occurs. This may be accomplished by chance inoculation, which requires considerable time and is uncertain, or by the use of soil or barnyard manure. The latter not only carries the desired organisms but also improves the physical conditions.

During the first year, the sulfates are just as toxic as are the chlorides, with sulfates, the toxicity decreases during the second and third year even where no treatment has been given to the soil. This raises the interesting

TABLE 10
Nitric nitrogen produced in 100 gm. of soil during 21 days' incubation with and without sodium chloride

TREATMENT	AMOUNT OF NITRIC NITROGEN FOUND		
	1921	July, 1922	August, 1923
	mgm.	mgm	mgm.
Untreated soil	40 6	56 3	53.2
Soil leached	46 2	53 9	62.0
Soil leached + 15 T. manure		53 2	109.5
Soil leached + soil extract		43 8	45.5
Soil + 2 per cent NaCl	5 6	9 5	15.7
Soil + 2 per cent NaCl leached	31 5	41 6	50.4
Soil + 2 per cent NaCl + 15 T. manure		1 4	15 5
Soil + 2 per cent NaCl leached + 15 T. manure		37 1	35.7
Soil + 2 per cent NaCl + soil extract		1 1	4.2
Soil + 2 per cent NaCl leached + soil extract		54 2	60 2
Soil + 1 per cent each NaCl, Na ₂ SO ₄	1 4	9 8	5 2
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	41 3	66 5	56.5
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + 15 T. manure		42 4	100.5
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + soil extract		47 6	49.5
Soil + 1 per cent each NaCl, Na ₂ CO ₃	4 9	4.5	7 7
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached	2.8	3 5	3 5
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + 15 T. manure		4.2	21.0
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + soil extract		2 8	2 8
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	1 4	33 5	23.8
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached	46 9	64 7	87.2
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + 15 T. manure		74.2	72.8
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached + soil extract		73 2	59.6
Corinne alkali soil	4 9	2 8	7.0
Corinne alkali soil + 15 T. manure		3 2	4.2
Corinne alkali soil leached	2.1	13 3	7.7
Corinne alkali soil leached + 15 T. manure		13 8	38.5
Corinne alkali soil + soil extract		1 4	3.8
Corrine alkali soil leached + soil extract		12 0	12.6

question: Has there developed within the soil an alkali-resistant strain of the nitrifiers? The mere leaching of the soil restores completely its nitrifying power, a thing which does not occur in the case of the chloride-carrying soil.

The addition of the soil extract has little value. This would be expected where the concentration of the alkali is not sufficient to destroy the nitrifying microflora. However, manure has a marked accelerating influence upon the

TABLE 11

Nitric nitrogen produced in 100 gm. of soil during 21 days' incubation with and without sulfates

TREATMENT	AMOUNT OF NITRIC NITROGEN FOUND		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil.....	40.6	56.3	53.2
Soil leached.....	46.2	53.9	62.0
Soil leached + 15 T. manure.....		53.2	109.5
Soil leached + soil extract.....		43.8	45.5
Soil + 2 per cent Na_2SO_4	1.4	21.0	47.6
Soil + 2 per cent Na_2SO_4 leached.....	47.6	41.3	56.0
Soil + 2 per cent Na_2SO_4 + 15 T. manure.....		56.0	14.3
Soil + 2 per cent Na_2SO_4 leached + 15 T. manure.....		63.0	98.7
Soil + 2 per cent Na_2SO_4 + soil extract.....		21.7	29.7
Soil + 2 per cent Na_2SO_4 leached + soil extract.....		52.8	47.2
Soil + 1 per cent each Na_2SO_4 , NaCl.....	1.4	9.8	5.2
Soil + 1 per cent each Na_2SO_4 , NaCl leached.....	41.3	66.5	56.5
Soil + 1 per cent each Na_2SO_4 , NaCl leached + 15 T. manure.....		42.4	100.5
Soil + 1 per cent each Na_2SO_4 , NaCl leached + soil extract.....		47.6	49.5
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3	4.2	3.2	4.8
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached.....	1.7	2.8	3.5
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 , leached + 15 T. manure.....		4.9	7.7
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 , leached + soil extract.....		40.9	1.4
Soil + 0.66 per cent each Na_2SO_4 , NaCl, Na_2CO_3	1.4	33.5	23.8
Soil + 0.66 per cent each Na_2SO_4 , NaCl, Na_2CO_3 leached.....	46.9	64.7	87.2
Soil + 0.66 per cent each Na_2SO_4 , NaCl, Na_2CO_3 leached + 15 T. manure.....		74.2	72.8
Soil + 0.66 per cent each Na_2SO_4 , NaCl, Na_2CO_3 leached + soil extract.....		73.2	59.6
Richland acre alkali soil.....	9.1	17.5	16.0
Richland acre alkali soil + 15 T. manure.....		7.0	27.0
Richland acre alkali soil leached.....	5.9	10.9	19.3
Richland acre alkali soil leached + 15 T. manure.....		6.0	26.9
Richland acre alkali soil + soil extract.....		25.2	11.2
Richland acre alkali soil leached + soil extract.....		13.7	10.8

nitrifying powers which probably comes from the improved physical condition of the soil, as it is apparent that aëration is much better in the manured than in the unmanured soil.

Sodium carbonate is more toxic when measured in terms of nitrification

than is either the chloride or the sulfate. The carbonate reduced nitrification to 7 per cent normal, the chloride to 12 per cent normal, and the sulfate to 47 per cent normal.

The percentage reduction of ammonification and nitrification for chlorides and sulfates is not far different, but the reduction of nitrification is much more pronounced in the presence of carbonates than is the reduction of ammonification. There are probably two factors involved: 1. Probably the nitrifying organisms are more sensitive to sodium carbonate than are the ammonifiers. 2. The nitrifying process is strictly aerobic, whereas ammonification may proceed under either aerobic or anaerobic conditions, and soil which contains sodium carbonate is in a very poor state of tilth.

Even after leaching, the soil shows very low nitrifying power. Neither the soil extract nor the manure is effective in restoring nitrification. It is evident that the soil must have some preliminary treatment before leaching and afterwards receive soil and manure—the first to inoculate, the second to improve tilth.

NITROGEN FIXATION

The nitrogen-fixing power of the soil was determined by the tumbler method. One-hundred-gram portions of the soil were weighed into sterile, covered tumblers. To each of these was added 1.5 gm. of lactose. This was carefully mixed and sufficient sterile distilled water added to bring the moisture up to 18 per cent. Each soil together with sterile blanks was incubated for 21 days at 28 to 30°C. after which the nitrogen was determined by the Gunning method revised to include nitrates (5). In every case four or more determinations were made on the soil from each pot. Hence, the results as given in the tables are the averages of eight determinations made on two similarly treated pots. In table 13 the results are given for the sodium-chloride-treated soil.

The direct leaching of the untreated soil has no measurable influence on the nitrogen-fixing power of the soil. The soil which had received 2 per cent sodium chloride and which was then leached showed increased nitrogen-fixing power. This is also true where the mixture of salts had been leached from the soil. The only fact which appears clear from these results is that the alkali-leached soil gains more rapidly in nitrogen when incubated than the untreated or alkali-containing soil. There is no evidence that the nitrogen fixers had been destroyed in the synthetic soils by the alkalies, as with one exception no benefit was derived from the use of manure or soil extract.

On the contrary, in the natural Corinne alkali soil, no gains of nitrogen were noted until this soil had been inoculated with soil extract containing the azotobacter.

The determination made in 1921 showed a loss of nitrogen in the sodium-sulfate-treated soil. In every determination made later the alkali-containing and the leached soil showed unmistakable evidence of gains in nitrogen. This is true in both the natural and synthetic alkali soil. There is no evidence that

the soil extract or manure is beneficial. It is evident that the nitrogen fixers survive this concentration of alkali, and when the salt is removed function more rapidly than they did before, probably on account of the absence of

TABLE 12
Nitric nitrogen produced in 100 gm. of soil during 21 days' incubation with and without sodium carbonate

TREATMENT	AMOUNT OF NITRIC NITROGEN FORMED		
	1921	July, 1922	August, 1923
	mgm.	mgm	mgm.
Untreated soil	40 6	56 3	53 2
Soil leached	46 2	53.9	62 0
Soil leached + 15 T. manure		53 2	109 5
Soil leached + soil extract		43 8	45 5
Soil + 2 per cent Na_2CO_3	6 3	0.7	3 5
Soil + 2 per cent Na_2CO_3 leached	2 1	2.4	3 2
Soil + 2 per cent Na_2CO_3 + 15 T. manure		1 4	1 7
Soil + 2 per cent Na_2CO_3 leached + 15 T. manure		3 5	1 4
Soil + 2 per cent Na_2CO_3 + soil extract		1 4	2 5
Soil + 2 per cent Na_2CO_3 leached + soil extract		4 5	1 7
Soil + 1 per cent each Na_2CO_3 , NaCl	4 9	4 5	7 7
Soil + 1 per cent each Na_2CO_3 , NaCl leached	2 8	3 5	3 5
Soil + 1 per cent each Na_2CO_3 , NaCl leached + 15 T. manure		4 2	21 0
Soil + 1 per cent each Na_2CO_3 , NaCl leached + soil extract		2 8	2 8
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4	4 2	3 2	4 8
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached	1.7	2 8	3 5
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached + 15 T. manure		4 9	7.7
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached + soil extract		40 9	1 4
Soil + 0.66 per cent each Na_2CO_3 , Na_2SO_4 + NaCl	1.4	33 5	23 8
Soil + 0.66 per cent each Na_2CO_3 , Na_2SO_4 + NaCl leached.	46 9	64 7	87.2
Soil + 0.66 per cent each Na_2CO_3 , Na_2SO_4 + NaCl leached. + 15 T. manure		74.2	72 8
Soil + 0.66 per cent each Na_2CO_3 , Na_2SO_4 leached + soil extract		73 2	59.6
Benson alkali soil	2.8	1 7	1.7
Benson alkali soil + 15 T. manure		2 4	1.0
Benson alkali soil leached	2.4	4.9	1.4
Benson alkali soil leached + 15 T. manure		1 7	2 8
Benson alkali soil + soil extract		1 1	4.5
Benson alkali soil leached and soil extract		1 1	3 2

competitive microorganisms. The results for the carbonates are given in table 15.

The sodium-carbonate-treated soil loses nitrogen about to the same extent

as the original soil, but when manure is added to such a soil the loss becomes very great, indicating a very perceptible denitrification. Such a soil, however, after being leached, although only 24 per cent of the carbonates had been

TABLE 13

Nitrogen fixed during 21 days in 100 gm. of soil to which had been added 1.5 per cent lactose with and without sodium chloride treatment

TREATMENT	AMOUNT OF NITROGEN FIXED		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil	-6.3	-0.8	10.5
Soil leached	0.0	-12.4	2.3
Soil leached + 15 T. manure		-4.4	3.5
Soil leached + soil extract		-9.6	3.2
Soil + 2 per cent NaCl	-5.6	1.4	-4.4
Soil + 2 per cent NaCl leached	2.8	4.2	2.3
Soil + 2 per cent NaCl + 15 T. manure		-16.4	-5.8
Soil + 2 per cent NaCl leached + 15 T. manure		-2.3	10.3
Soil + 2 per cent NaCl + soil extract		-8.5	-5.1
Soil + 2 per cent NaCl leached + soil extract		-9.2	-1.0
Soil + 1 per cent each NaCl, Na ₂ SO ₄	7.0	-4.2	12.4
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	9.8	1.1	6.5
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + 15 T. manure		-2.0	-1.4
Soil + 1 per cent each NaCl, Na ₂ SO ₄ + soil extract		-0.1	3.3
Soil + 1 per cent each NaCl, Na ₂ CO ₃	5.6	-0.2	-3.3
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached	11.2	5.8	8.9
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + 15 T. manure		13.8	5.1
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + soil extract		3.6	1.8
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	2.8	2.8	0.7
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached	10.0	3.3	1.1
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ + 15 T. manure		5.5	7.3
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ + soil extract		5.5	5.9
Corinne alkali soil	4.1	0.9	1.8
Corinne alkali soil + 15 T. manure		-0.3	4.2
Corinne alkali soil leached	0.0	-1.8	0.3
Corinne alkali soil leached + 15 T. manure		-2.6	0.3
Corinne alkali soil + soil extract		9.9	22.1
Corinne alkali soil leached + soil extract		1.4	1.0

removed, fixed marked quantities of nitrogen. This is also true in the natural alkali soil, in which sodium carbonate has been present for years. It is evident from these results that the nitrogen-fixing organisms are very resistant to alkali, as was earlier found by the direct tumbler method (4).

In order to obtain more definite information on this subject, leached and

unleached soils were inoculated into 100 cc. of Ashby solution, incubated 21 days, and the nitrogen was determined. The average results of eight determinations made in 1923 are given in table 16.

TABLE 14
Nitrogen fixed during 21 days in 100 gm. of soil to which had been added 1.5 per cent lactose with and without sulfates

TREATMENT	AMOUNT OF NITROGEN FIXED		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated	-6 3	-0 8	10 5
Soil leached	0 0	-12 4	2 3
Soil leached + 15 T. manure		-4 4	3.5
Soil leached + soil extract		-9 6	3 2
Soil + 2 per cent Na_2SO_4	-5 6	3 4	3 8
Soil + 2 per cent Na_2SO_4 leached	5.6	2 6	0 7
Soil + 2 per cent Na_2SO_4 + 15 T. manure		6.1	0 5
Soil + 2 per cent Na_2SO_4 leached + 15 T. manure		5 7	4.8
Soil + 2 per cent Na_2SO_4 + soil extract		6 0	1 4
Soil + 2 per cent Na_2SO_4 leached + soil extract		2 0	2 8
Soil + 1 per cent each Na_2SO_4 , NaCl	7 0	-4 2	12 4
Soil + 1 per cent each Na_2SO_4 , NaCl leached	9 8	1 1	6 5
Soil + 1 per cent each Na_2SO_4 , NaCl leached + 15 T. manure		-2 0	-1 4
Soil + 1 per cent each Na_2SO_4 , NaCl leached + soil extract		-0 1	3 3
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3	0 7	-0 9	-6 6
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached	1 4	7.7	13 3
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + 15 T. manure		-0 6	8 3
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + soil extract		5 2	3 4
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3	2 8	2 8	0.7
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached	10.0	3 3	1.1
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + 15 T. manure		5.5	7.3
Soil + 0.66 per cent each Na_2SO_4 , NaCl , Na_2CO_3 leached + soil extract		5 5	5.9
Richland acre alkali soil		3 9	8.3
Richland acre alkali soil + 15 T. manure		1 9	7.9
Richland acre alkali soil leached		6.1	4.2
Richland acre alkali soil leached + 15 T. manure		1.1	7.4
Richland acre alkali soil + soil extract		3.4	7.2
Richland acre alkali soil leached + soil extract		1.8	8 6

All of these soils when seeded into Asby solution fixed measurable quantities of nitrogen, thus substantiating the conclusion previously published (3), that the nitrogen-fixing organisms are much more resistant to soil alkalies than

are either the ammonifiers or nitrifiers. The results are more concordant with the solution than with the soil. It is significant that the leached soil invariably fixes more nitrogen than does the unleached soil. The fixation in the untreated

TABLE 15

Nitrogen fixed during 21 days in 100 gm. of soil to which had been added 1.5 per cent lactose with and without sodium carbonate

TREATMENT	AMOUNT OF NITROGEN FIXED		
	1921	July, 1922	August, 1923
	mgm.	mgm.	mgm.
Untreated soil	-6 3	-0.8	10 5
Soil leached	0 0	-12 4	2 3
Soil leached + 15 T. manure		-4 4	3.5
Soil leached + soil extract		-9 6	3 2
Soil + 2 per cent Na_2CO_3		-4.8	-1.0
Soil + 2 per cent Na_2CO_3 leached		4 3	4 2
Soil + 2 per cent Na_2CO_3 + 15 T. manure		-31 0	-5 2
Soil + 2 per cent Na_2CO_3 leached + 15 T. manure		3 6	8 3
Soil + 2 per cent Na_2CO_3 + soil extract		-1 8	2 8
Soil + 2 per cent Na_2CO_3 leached + soil extract		1 8	1.4
Soil + 1 per cent each Na_2CO_3 , NaCl	5.6	-0.2	-3 3
Soil + 1 per cent each Na_2CO_3 , NaCl leached	11 2	5 8	8 9
Soil + 1 per cent each Na_2CO_3 , NaCl leached + 15 T. manure		13 8	5 1
Soil + 1 per cent each Na_2CO_3 , NaCl leached + soil extract		3 6	1 8
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4	0 7	-0 9	-6 6
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached	1 4	7.7	13 3
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached + 15 T. manure		-0 6	8 3
Soil + 1 per cent each Na_2CO_3 , Na_2SO_4 leached + soil extract		5.2	3 4
Soil + 0.66 per cent each Na_2CO_3 , NaCl, Na_2SO_4	2 8	2 8	0 7
Soil + 0.66 per cent each Na_2CO_3 , NaCl, Na_2SO_4 leached	10.0	3 3	1.1
Soil + 0.66 per cent each Na_2CO_3 , NaCl, Na_2SO_4 leached + 15 T. manure		5.5	7.3
Soil + 0.66 per cent each Na_2CO_3 , NaCl, Na_2SO_4 leached + soil extract		5 5	5 9
Benson alkali soil		-0.9	-0.8
Benson alkali soil + 15 T. manure		-4 8	-2.3
Benson alkali soil leached		5.1	3 6
Benson alkali soil leached + 15 T. manure		11.0	7 0
Benson alkali soil + soil extract		0 4	0.9
Benson alkali soil leached + soil extract		5 1	3 6

and in the sodium-sulfate-treated leached soil is greater than in the natural soil. The fixation in the carbonate-containing soil is low but the results point clearly to the conclusion that nitrogen fixers survive this concentration of

alkali. Even the native alkali soil, in which the salts had been present for years readily induced nitrogen fixation after leaching.

Characteristic azotobacter films occurred in Ashby solutions inoculated with leached and non-leached normal soil, in leached soil which had contained sodium sulfate, and in the natural sodium sulfate soil (Richland acre soil). In fact, the membrane formed earlier and was darker in the presence of sodium sulfate than in its absence. The azotobacter was recovered from the solutions producing typical membranes. Thus far the author has failed to recover azotobacter from the other soils. Hence the question arises: What are the

TABLE 16

Nitrogen fixed in 100 cc. Ashby solution in 21 days. Inoculated with 1 gm. soil, spring 1924

TREATMENT	AMOUNT OF NITROGEN FIXED	EXCESS FIXED BY LEACHED
	mgm.	mgm.
Soil untreated	8 38	
Soil leached	10 03	1 92
Soil + 2 per cent NaCl	4.67	
Soil + 2 per cent NaCl leached	8.12	3 45
Soil + 2 per cent Na ₂ SO ₄	6 39	
Soil + 2 per cent Na ₂ SO ₄ leached	9 61	3.22
Soil + 2 per cent Na ₂ CO ₃	1.11	
Soil + 2 per cent Na ₂ CO ₃ leached	3 36	2.25
Soil + 1 per cent each NaCl, Na ₂ SO ₄	5.51	
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	6.15	0 64
Soil + 1 per cent each NaCl, Na ₂ SO ₄	5.51	
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	7 33	1 82
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃	5.83	
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached	8.49	2.66
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	6.07	
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached ..	6.21	0 14
Corinne alkali soil	1.40	
Corinne alkali soil leached	1.42	0.02
Richland acre alkali soil	4.62	
Richland acre alkali soil leached	4.39	-0.23
Benson alkali soil	0.51	
Benson alkali soil leached	3.76	3 25

organisms which are fixing nitrogen in these salt-containing soils? Is it possible that the anaerobic species *Clostridium* play an important rôle?

In table 17 is given the percentage loss or gain of nitrogen from the various soils. The total nitrogen was determined in the soil of each pot at the end of the leaching period. This in each case was taken as 100. The total nitrogen was redetermined at the end of two years. In the first column is given the percentage loss or gain from the specific soil during the two years; in the second column is given the total nitrogen removed in the crop; and in the third is given the gross percentage gain or loss of nitrogen. In interpreting these results one

must remember that one crop was the legume, crimson clover. Nodules were found on the clover grown on the untreated soil, on all of the soil which had received the soil extract, and on all of the soil containing the sulfates. In other words, the legume organisms were proved to be present in all of the soils which yielded positive tests for azotobacter.

The untreated soil lost nearly ten per cent of its nitrogen, and even after allowance has been made for the nitrogen removed in the plant there is still a loss of 2.47 per cent of the total nitrogen. This is not experimental error, as

TABLE 17
Loss or gain of total nitrogen in pot

TREATMENT	LOSS OR GAIN OF TOTAL N DURING 2 YEARS	TOTAL N REMOVED IN 2 CROPS	N RECOVERED AT END OF 2 YEARS IN SOIL AND CROP
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Soil untreated	-9.50	98.1	-2.47
Soil leached no alkali	-3.27	153.0	8.42
Soil + 2 per cent NaCl	5.07		5.07
Soil + 2 per cent NaCl leached	-2.90	99.9	5.35
Soil + 2 per cent Na ₂ SO ₄	5.66	8.1	6.29
Soil + 2 per cent Na ₂ SO ₄ leached	2.07	75.6	8.52
Soil + 2 per cent Na ₂ CO ₃	-17.98		-17.98
Soil + 2 per cent Na ₂ CO ₃ leached	0.99	4.5	1.51
Soil + 1 per cent each NaCl, Na ₂ SO ₄	0.66		0.66
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	2.52	65.6	9.17
Soil + 1 per cent each NaCl, Na ₂ CO ₃	6.90		6.90
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached	3.57	6.4	4.32
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃	3.63		3.63
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached	-9.23	5.6	-8.62
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	7.22		7.22
Soil + 0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ leached	15.27	24.0	12.81
Corinne alkali soil	7.07		7.07
Corinne alkali soil leached	17.77	10.8	18.77
Richland acre soil	2.77	0.9	2.80
Richland acre soil leached	11.09	0.9	11.12
Benson alkali soil	2.61		2.61
Benson alkali soil leached	34.53	5.4	36.30

each sampling was thorough, and represented the nitrogen in the soil. Moreover it manifested itself in the duplicate pots and would have to be recorded as an unknown loss, possibly denitrification. Only two other soils showed a loss, the one receiving sodium carbonate and the one receiving a mixture of sodium carbonate and sodium sulfate. In all but two cases the leached soil gained in nitrogen faster than did the unleached and in the case of the native alkali soil the gain was great.

In table 18 is given an inventory of the nitrogen of the various soils, calculated as pounds per acre.

Every soil except three registered a gain in total nitrogen, and all except five showed an actual gain in the soil. The leached in nearly every case made greater gains than did the unleached. The soil in which there was a mixture of the three salts gained more than did any of the other soils. The native alkali soil when leached, gained nitrogen rapidly. This was greatest where the predominating alkali was sodium carbonate and least where it was sodium sulfate. These results are significant in showing that alkali does actually

TABLE 18
Weight of total nitrogen in each acre

TREATMENT	AFTER LEACHING	LOSS THROUGH LEACHING		TOTAL LOSS OR GAIN FROM SOIL DURING 2 YEARS
		By difference	Calculated from recovered N	
	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Normal soil	5,397			-512
Normal soil leached	5,040	-357	-48	-165
2 per cent NaCl	5,040			256
2 per cent NaCl leached	4,839	-201	-223	-140
2 per cent Na ₂ SO ₄	5,090			288
2 per cent Na ₂ SO ₄ leached	4,687	-403	-112	97
2 per cent Na ₂ CO ₃	4,184			-752
2 per cent Na ₂ CO ₃ leached	3,578	-606	-1066	35
1 per cent each NaCl, Na ₂ SO ₄	4,334			29
1 per cent each NaCl, Na ₂ SO ₄ leached	4,435	101	-199	112
1 per cent each NaCl Na ₂ CO ₃	4,536			313
1 per cent each NaCl Na ₂ CO ₃ leached	3,830	-706	-379	137
1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃	4,662			169
1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached	4,133	-529	-438	-381
0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃	4,536			327
0.66 per cent each NaCl, Na ₂ SO ₄ , Na ₂ CO ₃ , leached	4,385	-151	-269	670
Corinne soil	4,738			335
Corinne soil leached	4,334	-404	-134	770
Richland acre	10,764			298
Richland acre leached	10,300	-464	-90	1142
Benson soil	2,066			54
Benson soil leached	1,210	-856	-507	418

cause a measurable gain of nitrogen in a soil and that the gain is in the same order as was found by the tumbler method.

The soil was shoveled direct from the field into the pots, hence the first accurate knowledge we have of the nitrogen content of each pot is that given in the first column. This was obtained by a careful analysis of the soil of each individual pot made at the close of the leaching period. The results given in the second column represent the loss due to leaching. They are based on the assumption that the two soils, leached and non-leached, contained soil of the same nitrogen content at the time they were put into the pots.

In column three is given the loss of nitrogen as calculated from the drain water. These two sets of results do not agree, nor should one expect them to for the following reasons:

1. Nitrogen fixation may have been greater in the leached than in the non-leached; hence, the leach water may carry nitrogen although the soil registers no loss in total nitrogen.

2. All of the nitrogen removed from the soil may not appear in the drain waters. The leaching soil filled with water has conditions ideal for denitrification, hence some of the nitrogen may disappear as gaseous nitrogen.

It is evident from the results that leaching has carried from the soil considerable nitrogen. This is greatest, as would be expected, where sodium carbonate is present.

The gain recorded in the second column where the sulfate and chloride were present should not be taken to indicate that no nitrogen was carried from the soil but that the gain due to fixation more than offset the loss due to leaching. Hence, the loss through leaching is the sum of the difference between the two soils plus the excess fixation in the leached soil.

The results given in the fourth column represent the gain or loss of nitrogen from these soils during two years. They are based on analysis of representative samples made in 1921 and again in 1923, and calculated to the dry basis.

It is certain from these results that some salts stimulate nitrogen fixation. The untreated soil registers a loss, whereas most of the alkali soils show a gain. The gain occurs in most cases more rapidly in the leached than in the non-leached soil, but even the alkali-containing soil shows measurable increases of nitrogen. The greatest gain is in the sulfate-containing soil. This is significant when it is recalled that it was from these soils that the *azotobacter* were recovered. Moreover it is clear that certain, as yet undetermined concentrations of various salts accelerate nitrogen fixation in soils.

CROP YIELDS FROM SOIL

The total dry weight and nitrogen in both the stalks and roots of the clover are given in table 19. Where no results are reported for a specific treatment it indicates that no crop grew on that soil. The results as reported are the averages for pots receiving the same treatment.

The leaching of the soil increased the total dry matter in the clover tops 38 per cent and in the roots 190 per cent. The total nitrogen is greatly increased, but the percentage of nitrogen in both roots and tops is decreased. The total dry matter is increased by both soil extract and manure over that in the leached soil; moreover, it is increased to the same extent by the soil extract as by the manure.

The soil containing 2 per cent of sodium chloride produced no crop. After being leached and treated with farmyard manure, the crop nearly equals that produced by the original soil. Here the manure is more effective than the soil extract, probably because of its ability to combine with and neutralize to a

TABLE 19
Weight of dry matter, total nitrogen, and percentage of nitrogen in clover roots and tops on various alkali soils

TREATMENT	ROOTS			TOPS		
	Weight dry matter	Total N	Total N	Weight dry matter	Total N	Total N
	mgm.	per cent	mgm	mgm	per cent	mgm.
Soil untreated	16 38	2 93	480	2 23	2 51	56
Soil leached	22 65	2 73	618	6 40	2 16	138
Soil leached + 15 T. manure	27 74	2 76	766	5 26	2 36	124
Soil leached + soil extract	28 26	2 80	791	4 91	2 13	105
Soil + 2 per cent NaCl, leached	13 99	3 10	434	2 86	2 46	70
Soil + 2 per cent NaCl, leached + 15 T. manure	15.25	3.23	493	3.11	2 67	83
Soil + 2 per cent NaCl, leached + soil ex- tract	10 50	3 84	403	2 45	2 92	72
Soil + 2 per cent Na ₂ SO ₄	0 24	3 31	8			
Soil + 2 per cent Na ₂ SO ₄ leached	17 55	2 82	495	3 35	2 21	74
Soil + 2 per cent Na ₂ SO ₄ + 15 T. manure	4 77	3 37	161	2 57	2 58	66
Soil + 2 per cent Na ₂ SO ₄ leached + 15 T. manure	19 47	3 48	330	1 73	2 61	45
Soil + 2 per cent Na ₂ SO ₄ leached + soil extract	19 65	3 92	770	1 06	2 60	28
Soil + 2 per cent Na ₂ CO ₃ leached	0 27	3 41	9	0 12	3 22	4
Soil + 2 per cent Na ₂ CO ₃ leached + 15 T. manure	1 69	4.56	76	0 74	2 51	19
Soil + 2 per cent Na ₂ CO ₃ leached + soil extract	0 73	4 58	33	0 22	3 40	8
Soil + 1 per cent each Na ₂ SO ₄ , NaCl leached Soil + 1 per cent each Na ₂ SO ₄ , NaCl leached + 15 T. manure	9 02	3.71	334	0 92	2.88	27
Soil + 1 per cent each Na ₂ SO ₄ , NaCl leached + soil extract	7.96	3.47	276	1.12	3 01	34
Soil + 1 per cent each Na ₂ SO ₄ , NaCl leached + soil extract	16.85	3 03	511	2 36	2.52	60
Soil + 1 per cent each Na ₂ CO ₃ , NaCl leached Soil + 1 per cent each Na ₂ CO ₃ , NaCl leached + 15 T. manure	0.01	5 35	1			
Soil + 1 per cent each Na ₂ CO ₃ , NaCl leached + soil extract	6.63	2 59	172	4.42	2.46	109
Soil + 1 per cent each Na ₂ CO ₃ , NaCl leached + soil extract	0.61	4.70	29	0.40	2 80	11
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached + 15 T. manure	3.50	4.10	144	0.42	3.06	13
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached + soil extract	0.80	4 07	33	0.38	2.70	10
Soil + 0.66 per cent each Na ₂ CO ₃ , Na ₂ SO ₄ , NaCl leached	1.80	4.34	78	0.40	3.62	15
Soil + 0.66 per cent each Na ₂ CO ₃ , Na ₂ SO ₄ , NaCl leached + 15 T. manure	6.88	3.27	225	0.69	2.49	17
Soil + 0.66 per cent each Na ₂ CO ₃ , Na ₂ SO ₄ , NaCl leached + soil extract	3.35	4.01	134	0.45	2.01	9
Corinne alkali soil leached	6.13	2.85	175	1.25	1.81	23

TABLE 19—*Continued*

TREATMENT	ROOTS			TOPS		
	Weight dry matter	Total N	Total N	Weight dry matter	Total N	Total N
	mgm.	per cent	mgm.	mgm.	per cent	mgm.
Corinne alkali soil leached + 15 T. manure	7 14	2 80	200	0 36	2 11	8
Richland acre soil	0 59	2 89	171			
Richland acre soil + 15 T. manure	0 41	3 59	15			
Richland acre soil leached	0 07	4 38	3			
Richland acre soil leached + 15 T. manure	0 20	4 81	9			
Benson alkali soil leached	0 35	2 08	7			
Benson alkali soil leached + 15 T. manure	2 40	3 97	95			
Benson alkali soil leached + soil extract	0 26	2 85	7			

limited extent the soluble salts. The percentage of nitrogen in the plants grown on the alkali soil is considerably higher than that in the plants grown on the normal soil.

The sodium-sulfate-carrying soil produced only one weak plant, but when this same soil received 15 tons of manure it produced only one-fourth as much as the normal soil. The leaching of the soil brought it back to normal. Leaching and the addition of manure increase the crop to above normal. Here also there is an increase in the percentage of nitrogen in the plants due to the alkali.

The sodium-carbonate-treated soil produced no crop even after leaching, and it was meager even after manure had been applied to the soil.

The mixture of sodium chloride and sodium sulfate prevented all growth, and leaching was ineffective in bringing it back to normal. The manure and soil extract, however, made the soil productive. Similar results were obtained where the mixture of the three salts was used. The results clearly indicate carbonate to be most toxic and sulfate least—the same order as was found for the bacteria.

It is quite evident from these results that the natural alkali soil is much harder to reclaim than is the synthetic alkali soil. This is due to recognized physical and chemical differences in these soils, and these results indicate that there are also biological differences.

The conclusions which seem warranted from these results are:

1. Black alkali soil is very difficult to reclaim; in these experiments none of the black alkali soil was restored to producing normal yields.
2. Organic manures are essential in reclaiming alkali soil even where leaching is used. Furthermore some alkali-containing soils can be made to produce fair crops by the addition of manure.
3. Crops grown on alkali soil are higher in nitrogen than are those grown on non-alkali soil.
4. The dry matter in the clover tops was 4.2 times that in the roots.
5. There was nearly five times as much nitrogen in the tops as in the roots. This is considerably higher in the hay than is the value reported by others (7).

The average results for barley are given in table 20.

In every case except two, the leached soil produced more barley than did

TABLE 20

Weight of dry matter, total nitrogen, and percentage of nitrogen in straw and barley grain on the various alkali soils

TREATMENT	STRAW			GRAIN		
	Weight dry matter	Total N	Total N	Weight dry matter	Total N	Total N
	mgm.	per cent	mgm.	mgm.	per cent	mgm.
Untreated soil	7 1	1.09	77	2 4	2.13	51
Soil leached	7 1	0 92	65	3 1	2 18	68
Soil leached + 15 T. manure	10 7	1.35	144	3 3	2 44	81
Soil leached + soil extract	8 3	0 83	69	1 9	2 31	44
Soil + 2 per cent NaCl	6 4	2 16	138	0 4	3 92	16
Soil + 2 per cent NaCl leached	8 5	0 96	82	2 9	2 41	70
Soil + 2 per cent NaCl + 15 T. manure	6 6	1 86	123	1 0	3 28	33
Soil + 2 per cent NaCl leached + 15 T. manure	9 3	1 07	100	4 2	2 45	103
Soil + 2 per cent NaCl + soil extract	5 0	1 43	72	2 0	2 78	56
Soil + 2 per cent NaCl leached + soil ex- tract	14 3	1 33	190	2 8	2 67	75
Soil + 2 per cent Na ₂ SO ₄	5 9	1 20	71	2 8	2 16	61
Soil + 2 per cent Na ₂ SO ₄ leached	15 1	2 20	332	2 8	3 12	87
Soil 2 per cent Na ₂ SO ₄ + 15 T. manure	15 5	2 20	341	3 2	3 46	111
Soil + 2 per cent Na ₂ SO ₄ leached + 15 T. manure	17 8	1 68	299	2 4	2 76	66
Soil + 2 per cent Na ₂ SO ₄ + soil extract	15.5	1 85	287	2 3	3 18	73
Soil + 2 per cent Na ₂ SO ₄ leached + soil ex- tract	17 5	2 10	368	2 6	2 85	74
Soil + 2 per cent Na ₂ CO ₃ leached	2 4	1 33	32	0 2	3 56	7
Soil + 2 per cent Na ₂ CO ₃ leached + 15 T. manure	13 3	1 71	227	2.3	2 81	65
Soil + 2 per cent Na ₂ CO ₃ leached + soil ex- tract	6 1	1 75	107	2 1	2.01	42
Soil + 1 per cent each NaCl, Na ₂ SO ₄	6 3	1 55	98	1 2	2 92	35
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached	11 2	1 33	149	2 6	2.63	68
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + 15 T. manure	9 3	1 54	143	2 0	2.76	55
Soil + 1 per cent each NaCl, Na ₂ SO ₄ leached + soil extract	7 4	0 90	67	2 8	2 17	61
Soil + 1 per cent each NaCl, Na ₂ CO ₃	1 6	2 24	36	0 1	2 62	3
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached	9.1	1.45	132	0 6	3 23	19
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + 15 T. manure	11.6	1 18	137	2 5	2.50	63
Soil + 1 per cent each NaCl, Na ₂ CO ₃ leached + soil extract	11 27	0 93	105	3.6	2.23	80
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃	3.2	3.17	101			
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₃ leached	1.7	1.35	23	1.8	2.46	44
Soil + 1 per cent each Na ₂ SO ₄ , Na ₂ CO ₄ leached 15 T. manure	12 2	1 27	154	3.4	2 45	83

TABLE 20—Continued

TREATMENT	STRAW			GRAIN		
	Weight dry matter	Total N	Total N	Weight dry matter	Total N	Total N
	mgm	per cent	mgm.	mgm.	per cent	mgm.
Soil + 1 per cent each Na_2SO_4 , Na_2CO_3 leached + soil extract	8 8	0 65	52	2 9	2.00	58
Soil + 0.66 per cent each NaCl , Na_2SO_4 , Na_2CO_3	10 4	1 45	151	2 4	2 43	58
Soil + 0.66 per cent each NaCl , Na_2SO_4 , Na_2CO_3 , leached.	13 1	1 90	249	3.3	2 52	83
Soil + 0.66 per cent each NaCl , Na_2SO_4 , Na_2CO_3 + 15 T. manure	20 6	1 99	410	1 2	3 46	42
Soil + 0.66 per cent each NaCl , Na_2SO_4 , Na_2CO_3 + soil extract	15 8	1 91	302	3 9	2 60	101
Corinne soil			No crop			
Corinne soil + 15 T. manure	8 0	1 32	106	2 1	2.74	58
Corinne soil leached	13 5	1.15	155	2 90	2 46	71
Corinne soil leached + 15 T. manure	16.0	1 73	277	2 7	2 22	60
Corinne soil + soil extract	1 6	1 98	21			
Corinne soil leached + soil extract	15 4	2 90	447			
Richland acre soil	3 7	2 12	78	0 3	3 37	10
Richland acre soil + 15 T. manure	9 5	2 33	221	0 9	3 32	30
Richland acre soil leached	0 6	3 48	21			
Richland acre soil leached + 15 T. manure	9 5	2 48	236	0.6	2 92	18
Richland acre soil + soil extract	0 5	1 94	10	1 3	2 74	36
Richland acre soil leached + soil extract	7 7	1 38	106	3 2	2 65	85
Benson alkali soil			No crop			
Benson alkali soil + 15 T. manure			No crop			
Benson alkali soil leached.	11 7	0 99	116	2 0	2 42	48
Benson alkali soil leached + 15 T. manure.	20 9	1 80	376	0.9	2 58	23
Benson alkali soil + soil extract			No crop			
Benson alkali soil leached + soil extract	22 6	1 13	255	3 3	2 65	87

the unleached soil. There was also considerably more nitrogen removed in the grain from the leached than from the unleached soil. It is evident that the change produced in the bacterial activities of the soil by leaching is reflected in the crop yields. This increase is augmented by the manure, but no beneficial effect can be attributed to the inoculation. In reality, the results point to a depression caused by the soil extract, thus seeming to indicate that the soil extract has carried back into the soil the limiting factor of bacterial activity and growth.

Two per cent of sodium chloride retards but does not prevent plant growth. However, it does reduce the proportion of grain to straw and results in a straw and grain both of which are richer in nitrogen. Leaching increases the yield and reduces the percentage of nitrogen carried by the crop. Manure applied to the unleached soil has little value, but when applied to the leached soil is

very effective is increasing productivity. Here again very little influence could be attributed to the soil extract.

The sodium-sulfate-treated soil produced slightly less than the normal soil, but when leached and especially when treated with manure it was more productive than the untreated soil. Here again the soil extract is less effective than the manure.

The sodium-carbonate-treated soil produced no crop, and even after it had been leached, the yield was low until manure had been applied. The soil extract is beneficial but not to the same extent as is the manure. The percentage nitrogen in the crop is increased by sodium carbonate and sodium sulfate.

The toxicity of the mixture of the salts is less than that of the single salts. Hence, there appears to be an antagonistic action of the one salt toward the other.

The soil receiving the combination of the three salts was as productive as the untreated soil, and after being leached, and especially after treatment with manure or soil extract, produced a much greater crop than did the untreated.

It is evident from these results that the addition of the alkali to a soil and then the removal by leaching have increased its productivity and also modified the soil to produce a crop richer in nitrogen. Moreover, it is very evident from these results that farmyard manures must play a very prominent part in the reclaiming of alkali soil. This is brought out even more strikingly with the naturally occurring than with the artificially produced soil, for often the mere presence of manure is sufficient to determine whether there be a crop. This in some cases was true for both the leached and non-leached alkali soils. The Corinne soil in the absence of manure produced no crop; in the presence of manure the yield equalled that on the college farm soil. When this was leached and treated with manure or soil extract the crop yield was doubled. Similar results were obtained on the Richland acre soil. The Benson alkali which is heavily impregnated with black alkali became productive only after leaching. After leaching, both the soil extract and manure is highly efficient in increasing productivity.

Conclusions drawn from these results can be accepted as only tentative, indicating the plan which should be followed in future work in both pot and field experiments. They are published at this time, as the nature of the work has been such that further cropping on these soils is impracticable. The large quantities of soil removed in the sampling has left an insufficient amount for normal plant growth. Although it is realized that the results in many cases are inconclusive and bring out strikingly the need of more refined bacteriological methods to measure the biological differences of various soils, yet they conclusively prove that in the reclaiming of alkali soil there is a biological as well as a chemical and physical problem and that careful biological studies of soil alkali problems will yield results just as valuable as are now being obtained by recent chemical investigations.

CONCLUSIONS

The soils used in this work were three natural alkali soils: one in which chlorides predominated, a second in which the sulfates predominated, and a third which was heavily impregnated with sodium carbonate. A productive calcareous silt loam, which was made into an alkali soil by various treatments with sodium chloride, sulfate, and carbonate, was also used.

These were leached in 2-gallon jars to remove as much as practicable of the soil alkali. The soils were then sampled and manured or inoculated and cropped first to clover and afterwards to barley.

The leaching of the normal soil increased the bacterial activities and the productiveness.

Two per cent of sodium chloride decreased the bacterial numbers 11 per cent. Leaching caused an increase of 38 per cent over the normal soil. The results for the soil receiving 2 per cent of sodium sulfate were within the experimental error of those in the untreated soil.

Two per cent sodium carbonate reduced the numbers 24 per cent. Leaching increased them 830 per cent. That is, in all cases the leaching of the alkali and non-alkali soils increased the number of microorganisms.

Two per cent of sodium chloride reduced ammonification to 10 per cent of normal; sodium carbonate to 41 per cent of normal; and sodium sulfate to 52 per cent of normal. Leaching in many cases increases ammonification to above that occurring in the normal soil. Soil extract and manure were very effective in restoring ammonification in alkali soils.

Sodium carbonate is more toxic when measured in terms of nitrification than is either the chloride or the sulfate. The carbonate reduced nitrification to 7 per cent normal, the chloride to 12 per cent normal, and the sulfate to 47 per cent normal. Nitrification was very low even after leaching but was increased by both soil extract and manure, thus showing that the soil needs inoculation after the removal of the alkali.

Inconclusive results were obtained in nitrogen fixation by the tumbler method but very uniform results were obtained where the soil was seeded into Ashby media. Here alkalies retarded the fixation of nitrogen and in every case there was an increase caused by leaching.

Test of the soil at the beginning and end of the experiment showed that in most cases measurable quantities of nitrogen had been fixed and some of the alkalies, especially the sulfate, increased the rate of gain of nitrogen in the soil. This was especially true in the case of a natural alkali soil very high in sulfates.

The results indicate that the nitrogen fixing organisms are more resistant to soluble salts than other beneficial microorganisms.

The leaching of the soil increased the crop yield, and some of the alkali very markedly increased the nitrogen content of the plants.

It is evident from these results that organic manures are essential for the restoration of alkali soils to a high state of productivity. Benefits will often

result from the proper inoculating of alkali soils which are being reclaimed by leaching.

REFERENCES

- (1) Association of Official Agricultural Chemists. 1920. Official and Tentative Methods of Analysis, p. 8. *Assoc. Off. Agr. Chem.*, Washington, D. C.
- (2) GREAVES, J. E. 1916. The influence of salts on the bacterial activities of the soil. *Soil Sci.* 2: 443-480.
- (3) GREAVES, J. E. 1922. Influence of salts on bacterial activities of the soil. *Bot. Gaz.* 73: 161-180.
- (4) GREAVES, J. E., CARTER, E. G., AND LUND, YEPPA. 1922. Influence of salts on azoification in soil. *Soil Sci.* 13: 481-499.
- (5) GREAVES, J. E., AND HIRST, C. T. 1917. Some factors influencing the quantitative determination of nitric nitrogen in soil. *Soil Sci.* 4: 179-205.
- (6) GREAVES, J. E., HIRST, C. T., AND LUND, YEPPA. 1923. The leaching of alkali soil. *Soil Sci.* 16: 407-426.
- (7) HOPKINS, C. G. 1910. Soil Fertility and Permanent Agriculture, p. 218. New York.
- (8) YODER, P. A. 1904. A new centrifugal soil elutriator. *Utah Agr. Exp. Sta. Bul.* 89: 47.

THE NORMAL MOISTURE CAPACITY OF SOILS

CHARLES F. SHAW¹

University of California

Received for publication June 19, 1926

When water is applied to the surface of a more or less dry soil, it flows down through the interstices of the soil mass wetting the soil grains over which it passes. If the soil be *uniform in texture and structure and the quantity of water just sufficient to wet it to the full depth of uniformity*, or to a change in the soil character, *the amount of water retained on any of the soil grains throughout the entire depth will be approximately the same.*

If the quantity of water be insufficient to wet the full depth of uniform soil, the moisture content will be approximately uniform from the surface to within a few inches of the bottom of the wetted soil, where a rapid decrease occurs, the amount tapering down sharply to the dry soil. The depth to which the moisture penetrates is readily discernible, the change or break from wet to dry soil being very abrupt, and usually marked by a distinct change in color.

The flow of water downward through the soil mass is the result of two forces, gravity and an inverted "capillary movement" or film force. As the water moves down through the soil, a part is absorbed and held by the colloidal material present and an additional amount is held as thin films surrounding the soil grains with thicker waist-like accumulations where the grains touch each other. The downward pull of the "capillary" or film forces, augmented by the pull of gravity, reduces these films to a minimum thickness, and the curve of the water-air surfaces is sharp at points where the soil grains are in contact. When all the water is used in wetting the soil to this degree, further movement through the soil of water in the liquid phase is exceedingly limited and the moisture condition becomes essentially static.

LITERATURE

There is a voluminous literature dealing with soil moisture in general, usually with the "capillary rise" and drainage phenomena, but with relatively little bearing directly on downward percolation and the resulting distribution of the soil moisture.

Rotmistrov (5) has discussed this distribution and given field data showing relatively uniform moisture content within soil horizons that apparently have uniform characteristics.

¹ Professor of Soil Technology.

He gives no information on the texture or structure of these soil horizons, however, and the relation between the moisture holding capacity and the observed moisture can not be checked.

Burr (2, 3) has dealt with the problem in several papers, nearly all based on field observation and study where, however, the final moisture distribution is complicated by the presence of growing vegetation.

Alway and McDole (1) have discussed the downward movement of moisture and the resulting distribution in soils differing in textures from sands to silt loams. Short tubes (14.22 inches) were used and the distribution is reported in three and four sections after a period of five days or less. They concluded that "at the end of five days equilibrium had been practically attained in the fine-textured soils, but in the coarser one this was far from being the case. The coarser the soil the more slowly was equilibrium reached."

Scofield (6) has added to the data and discussion, as have many other investigators who have touched upon the problem incidental to other studies.

Israelsen and West (4) have studied the problem in the field, using large applications of water, with results that generally agree with the findings reported in this paper. They state that, "ten days after the heavy irrigations were applied by the authors each of the plots held the same amount of available water, namely $1\frac{1}{2}$ inches per foot in the upper 6 feet." In their conclusions, however, they state: "In the opinion of the authors, the downward movement of the water must continue until equilibrium is established with the water table. Moisture determinations that were made throughout the summer after the 10-day tests showed a continuous but very slow downward movement of water, thus confirming this opinion."

Widtsoe and McLaughlin (9) in their studies on the Greenville soil have recognized a relatively static soil moisture distribution for this soil at 12.68 per cent moisture, which they termed the "point of lento-capillarity" and which is well above the hygroscopic coefficient of 4.33 per cent. They did not, however, associate this with what they termed the "optimum capillary capacity or field water capacity" of this soil, which was placed at 18 per cent or about 5.3 per cent higher than the point of lento-capillarity. In the experiments conducted in our laboratories, there is strong evidence that at the "normal moisture capacity" which would correspond to Widtsoe's "field water capacity," the soil moisture condition is practically static.

Shantz (7) in a recent contribution has ably set forth a theory and conclusion essentially identical with that given herewith, but without any supporting data, although in earlier publications he had indirectly given much supporting evidence.

Vejh Meyer (8), in the results of exhaustive field investigations extending over several seasons, has given ample evidence of the soundness of the theories when applied to field practice in irrigated regions.

The problem has been under investigation in our laboratories for over ten years, and from the data thus accumulated the following evidence is submitted.²

EXPERIMENTAL WORK

The soil

Various soils ranging in texture from medium sands to clay loams were used during the experiment. The movement of water through the heavier-textured soils was so slow that the results were unsatisfactory and inconclusive and the more extended experiments were conducted with medium-textured soils. All the results herein reported were obtained by using a Yolo fine sandy loam soil

² Acknowledgment is due to B. F. Monroe, to E. V. Winterer, and to the many graduate students who have contributed to the work.

containing approximately 8.5 per cent clay, 11.5 per cent silt, and 70 per cent fine and very fine sand. As the experiments were continued over a period of several years, succeeding quantities of soil varied slightly in mechanical composition. The results given in tables 1 and 2 were from soils having a moisture equivalent just above 14 per cent, and those in tables 4 and 5 from a soil having a moisture equivalent of 15.88 per cent.

Methods

Brass tubes 4 inches in diameter and of 18-inch and 36-inch lengths were used for most of the work, while galvanized iron tubes 6 and 8 inches in diameter and from 4 to 8 feet long were used for a few tests.

The brass tubes were filled on the propeller type of compactor, giving a very uniform compaction of the soil throughout the full length of the container. The larger tubes were filled by carefully pouring in the soil, jarring to gain compaction. The distribution within the larger tubes was much less satisfactory, considerable stratification of the soil occurring in some cases.

Water was applied at the surface in quantity sufficient to give the desired depth of irrigation, a depth or head of 1 to $1\frac{1}{2}$ inches usually being maintained until all the water had been applied. Drip and sprinkling were used as means of application in certain cases when it was desired to eliminate the effect of "head." As soon as the water had disappeared from the surface the tubes were closed with a closely fitting, but not air-tight, cover in order to retard or prevent evaporation.

When a specified period of time had elapsed, the soils were sampled by removing, with a specially devised sampling tool, all the soil to the desired depth. In most cases the soils were sampled by 1-inch sections. The soil was quickly mixed, sub-sampled, and weighed into dishes and dried for from two to five days at 100°C. All results are reported at percentages of moisture based on the oven-dry weight of the soil.

EXPERIMENTAL DATA

Experimental results are given from only a few of the numerous tests made, selected to cover the several points discussed. Table 1 and figure 1 show the moisture distribution 5 days after irrigation of 18-inch tubes when 2, $2\frac{1}{2}$, 3 and $3\frac{1}{2}$ inches of water were applied. The soils were sampled by 1-inch sections and the results are the mean of duplicate tubes.

Although the depth of penetration varied in direct relation to the amount of moisture applied and the quantity above the taper was rather close in all the tubes, it was evident that downward movement had not ceased and that a longer period of time was needed before equilibrium would be established.

Table 2 and figure 2 show results where the amount of water applied was the same, but the time varied. Five tubes were used, irrigated with $2\frac{1}{2}$ inches of water, and allowed to stand 5, 7, 11, 21 and 28 days, protected from evapora-

tion as indicated above. The total penetration varied from 11 to 14 inches, with tapers of from four to five inches. All the tubes show a decrease in the amount of moisture from the surface downward, the slope becoming steeper as the time increased.

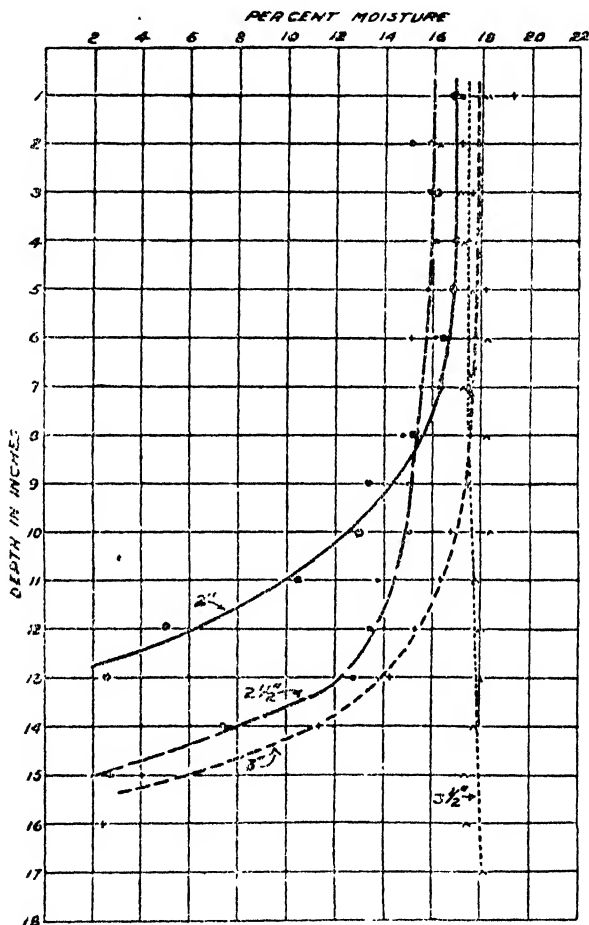


FIG. 1. EFFECT OF INCREASED AMOUNTS OF WATER. SAMPLED FIVE DAYS AFTER APPLICATION

———— = 2.0 inch depth of water applied; — — — = 2.5 inch depth of water applied;
 - - - - = 3.0 inch depth of water applied; - · - · - = 3.5 inch depth of water applied.

Because of the shallow depths of the soil, the uniformity of distribution in the 21- and 28-day tubes was considered as not sufficiently conclusive and for this reason other tests using longer tubes were undertaken. Brass tubes 4 inches in diameter and 36 inches long were used and irrigations giving 6, 7, and 8 inches of water applied. One set of tubes was sampled at the end of 24 days,

TABLE 1
Effect on moisture distribution of increased amounts of water

DEPTH OF SOIL	DEPTH OF WATER APPLIED			
	2.0 inches	2.5 inches	3.0 inches	3.5 inches
<i>inches</i>	<i>per cent</i> *	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	16.81	17.00	19.19	18.13
2	15.84	14.94	17.19	16.05
3	16.00	15.70	17.60	17.02
4	16.90	16.00	17.80	17.20
5	16.86	15.70	18.11	17.60
6	16.66	16.03	15.01	18.12
7	16.16	15.41	17.85	17.43
8	15.10	14.78	17.75	18.13
9	13.36	15.00	17.72	19.22
10	13.00	15.05	16.79	18.49
11	10.37	13.85	16.30	17.82
12	5.25	13.33	15.11	18.02
13	2.44	12.91	14.15	18.02
14	7.32	11.30	17.85
15	2.45	3.96	17.22
16	2.30	17.53
17	18.09

* Based on oven-dry weight of soil.

TABLE 2
Effect of time on moisture distribution

DEPTH OF SOIL	TIME ELAPSED BEFORE SAMPLING				
	5 days	7 days	11 days	21 days	28 days
<i>inches</i>	<i>per cent</i> *	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	21.25	18.59	19.02	15.95	15.04
2	18.45	17.10	16.38	13.72	14.78
3	18.27	16.71	15.80	14.25	14.73
4	17.81	17.37	16.10	14.15	14.04
5	18.33	17.16	16.29	14.07	14.53
6	17.78	16.14	15.39	14.34	14.24
7	17.87	16.34	15.67	14.54	14.44
8	16.70	15.75	14.72	14.04	14.14
9	16.41	14.58	14.07	13.71	13.58
10	14.87	13.48	13.34	13.00	13.08
11	13.48	12.72	12.18	11.94	12.66
12	11.45	11.79	3.65	9.97	12.07
13	3.72	4.60	7.93	10.04
14	2.90	2.92	4.65
15	2.59	3.17	2.21	3.68

* Based on oven-dry weight of soil.

and a second set after 54 days. Table 3 gives the moisture content by inches, and figure 3 shows the moisture distribution at the end of 54 days.

It is evident that the moisture had not reached a uniform distribution or a state of equilibrium in any of the tubes, although that condition is approximated after 54 days in the tubes receiving 6 and 7 inches of water. The water had reached the bottom of the tubes which received the 7- and 8-inch irrigations,

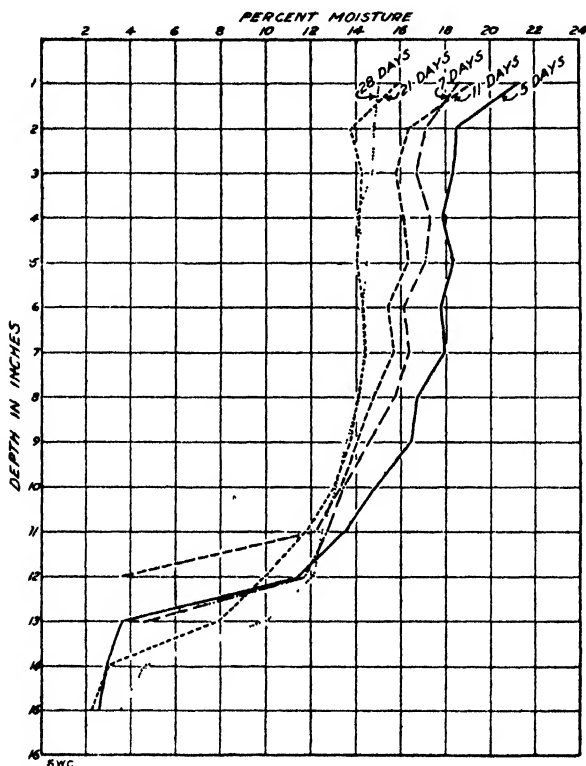


FIG. 2. EFFECT OF TIME ON MOISTURE DISTRIBUTION WHEN 2.5 INCHES OF WATER AS APPLIED

———— = 5 days; - - - - = 7 days; - - - - = 11 days; - · - · - = 21 days; · · · · · = 28 days.

making it evident that not over 6 inches of water could be held within the 36-inch soil column without wetting the soil to its entire depth.

The distribution as affected by a still more prolonged period is shown in table 4 and figure 4, which give the results obtained from tubes that received 6 inches of irrigation water and stood for 24, 54, 89, and 123 days.

The soil moisture had not ceased its downward flow in 89 days, but by 123 days the distribution closely approaches uniformity to a depth of 24 inches where the taper appears to begin. The average content of moisture (table 5)

from the surface to the twenty-fifth inch (excluding the eleventh inch) is 16.79 ± 0.365 , expressed as percentage of the dry weight. There appears to

TABLE 3
Distribution of water—effect of time and quantity

DEPTH	SIX INCHES		SEVEN INCHES		EIGHT INCHES	
	24 days	54 days	24 days	54 days	24 days	54 days
<i>inches</i>	<i>per cent*</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	21.70	19.04	20.50	21.10	23.53	22.70
2	19.52	18.15	19.82	19.33	21.38	20.50
3	19.73	18.38	19.64	18.96	20.54	20.32
4	20.84	16.48	20.02	19.05	20.85	20.32
5	19.61	18.05	19.48	18.96	21.03	20.50
6	19.52	17.53	19.84	18.95	21.15	20.70
7	20.53	18.28	20.43	19.41	21.32	20.61
8	22.53	18.88	20.01	19.48	21.10	21.45
9	..	18.50	19.82	19.39	21.72	21.15
10	20.04	18.56	20.70	19.63	22.20	21.15
11	20.67	18.93	20.68	19.23	21.47
12	20.79	18.41	20.70	19.57	21.63	21.50
13	20.64	18.15	21.28	19.55	21.30	21.82
14	20.75	18.75	21.38	19.60	22.38	22.25
15	20.45	16.95	21.10	19.63	22.42	21.88
16	20.28	18.70	21.62	19.42	21.75	22.10
17	20.30	17.85	21.22	19.63	22.27	22.33
18	19.96	18.52	16.50	19.41	22.58	22.47
19	19.05	18.52	21.10	19.83	22.45	22.70
20	17.94	18.25	21.11	19.15	22.23	21.28
21	18.65	17.42	20.80	19.65	22.40	21.99
22	18.32	17.49	20.28	19.21	22.23	22.02
23	18.35	17.28	20.18	19.13	22.30	22.71
24	17.42	16.80	14.35	18.95	21.70	22.20
25	16.74	16.40	19.42	18.83	20.72	21.58
26	15.90	15.63	18.90	18.52	22.22	21.42
27	14.35	15.62	19.05	18.61	20.89	18.24
28	12.46	15.01	18.53	18.49	20.89	21.98
29	5.91	14.21	17.68	18.31	20.18	22.28
30	3.33	13.68	16.40	14.20	19.69	21.65
31	9.25	15.95	13.75	19.92	21.65
32	5.23	14.51	13.74	19.86	22.10
33	4.43	10.20	13.48	20.42	22.81
34	3.72	4.32	13.60	20.08	22.42
35	16.90	17.65	21.90
36

*Based on oven-dry weight of soil.

be a slightly larger amount of moisture between the twelfth and seventeenth inches, but the largest variation, as shown in table 5, is only $+0.53$ as

compared to the average, or about 3 per cent of the average amount of moisture present.

That there was little or no evaporation loss and that the differences of distribution are due to moisture movements within the soil mass was shown by measurements of the areas within large scale graphs showing the distribution in the 24-, 54-, 89-, and 123-day tubes, where every point was located, and there

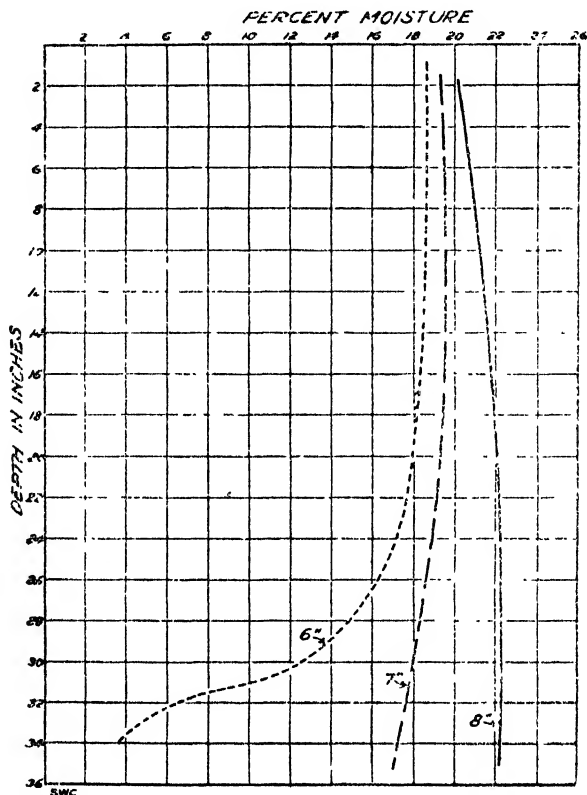


FIG. 3. EFFECT OF QUANTITY ON DISTRIBUTION OF WATER, SAMPLED 54 DAYS AFTER APPLICATION

———— = 8-inch application; - - - - = 7-inch application; = 6-inch application.

was no "smoothing" of the curve as in *A*, *B*, *C*, and *D* in figure 4. The area within *A* (24-day) was taken as 100, *B* (54-day) was found to be 98.5, *C* (89-day) to be 99.9, and *D* (123-day) to be 99.5. As these figures were obtained from different tubes of soil after standing for long periods of time the agreement is surprisingly close.

It is believed that the moisture at the end of 123 days had approximately reached equilibrium insofar as flow in the liquid phase is concerned, and that

any further movement would have to take place in the vapor phase. It is possible that a very slow downward flow might continue for some time, slightly

TABLE 4

Distribution of water in tubes receiving a 6-inch irrigation, after prolonged periods of adjustment

DEPTH	TIME			
	24 days	54 days	89 days	123 days
<i>inches</i>	<i>per cent*</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	21.70	19.04	17.64	17.32
2	19.52	18.15	16.95	16.72
3	19.73	18.38	17.02	16.11
4	20.84	16.48	16.93	16.67
5	19.61	18.05	17.25	16.72
6	19.52	17.53	17.25	16.45
7	20.53	18.28	17.47	17.26
8	22.53	18.88	17.44	17.32
9	18.50	17.41	16.60
10	20.04	18.56	17.57	16.82
11	20.67	18.93	17.86	15.24
12	20.79	18.41	16.82	17.16
13	20.64	18.15	18.14	17.00
14	20.75	18.75	18.05	17.13
15	20.45	16.95	17.78	16.84
16	20.28	18.70	18.24	17.32
17	20.30	17.85	17.45	17.23
18	19.96	18.52	17.58	16.83
19	19.05	18.52	17.46	16.69
20	17.94	18.25	16.92	16.39
21	18.65	17.42	17.09	16.54
22	18.32	17.49	17.02	16.68
23	18.35	17.28	16.65	16.32
24	17.42	16.80	16.57	16.16
25	16.74	16.40	16.00	15.72
26	15.90	15.63	15.90	15.79
27	14.35	15.62	15.55	15.62
28	12.46	15.01	15.23	15.17
29	5.91	14.21	14.83	14.88
30	3.33	13.68	14.34	14.42
31	9.25	13.53	13.95
32	5.23	12.64	13.62
33	4.43	11.09	12.73
34	3.72	8.12	11.07
35	6.06	8.29
36	3.92	6.62

* Based on oven-dry weight of soil.

reducing the moisture in the upper portion and increasing the depth of penetration. Table 4 shows that at the depth of 27 inches, the moisture content

has remained approximately constant after the fifty-fourth day, at a content of 15.62, 15.55 and 15.62 per cent. This evidently is a "critical" moisture point and may represent the content at equilibrium toward which the moisture is working.

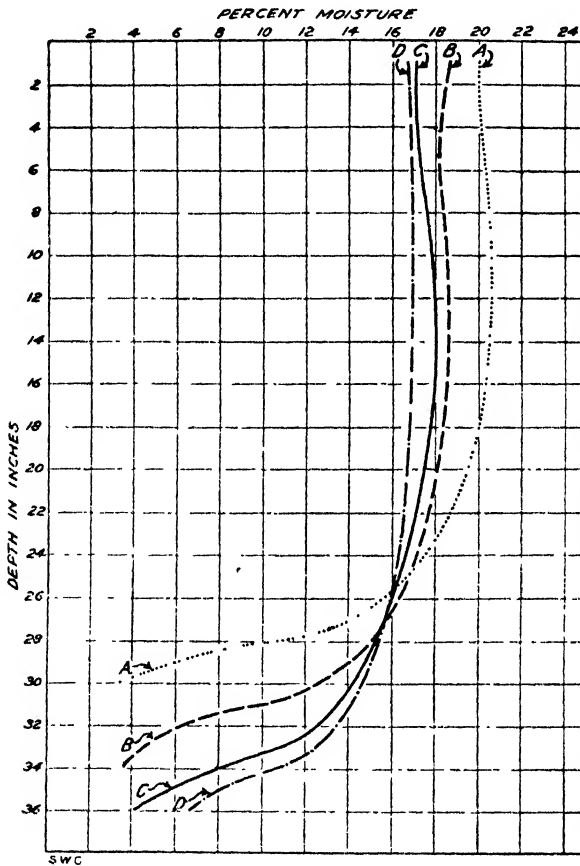


FIG. 4. DISTRIBUTION OF WATER IN TUBES RECEIVING A 6-INCH IRRIGATION, AFTER PROLONGED PERIODS OF ADJUSTMENT

..... A = Distribution after 24 days; — — — B = Distribution after 54 days;
 ————— C = Distribution after 89 days; - - - - D = Distribution after 123 days.

The average moisture equivalent of the soils used in this test was found to be 15.88 ± 0.34 , the variations of the individual tests being shown in table 6.

The close agreement of this moisture equivalent of 15.88 with the content of water at the twenty-seventh inch of 15.62 suggests that the normal moisture content of this soil when at equilibrium with the film and gravity forces is almost identical with the moisture equivalent. The average content at the end of 123 days, 16.79 per cent, is only 1.057 times the moisture equivalent; or

TABLE 5

Moisture content of 123-day tube above taper or to a depth of 24 inches, with averages and variations

DEPTH	PER CENT	VARIATION FROM AVERAGE
1	17.32	+0.53
2	16.72	-0.07
3	16.11	-0.68
4	16.67	-0.12
5	16.72	-0.07
6	16.45	-0.34
7	17.26	+0.47
8	17.32	+0.53
9	16.60	-0.19
10	16.82	+0.03
11	(15.24*)
12	17.16	+0.37
13	17.00	+0.21
14	17.13	+0.34
15	16.84	+0.05
16	17.32	+0.53
17	17.23	+0.44
18	16.83	+0.04
19	16.69	-0.10
20	16.39	-0.40
21	16.54	-0.25
22	16.68	-0.11
23	16.32	-0.47
24	16.16	-0.63
Average.	16.79	±0.365
Coefficient of Variation		2.15

* Not included in average.

TABLE 6

Moisture equivalents on soil from tubes

VARIATION
15.78 -0.10
15.65 -0.23
16.20 +0.32
15.28 -0.60
16.27 +0.39
15.80 -0.08
16.21 +0.33
Average = 15.88 ±0.34
Coefficient of Variation = 2.14

expressing it the opposite way, the moisture equivalent is 94.6 per cent of the average moisture content. The soils packed in the tubes had a volume weight of 1.315 and a pore space of 50.4 per cent. At the average moisture content at the end of 123 days, 43.8 per cent of the pore space was occupied by moisture, whereas at the moisture equivalent point, 41.4 per cent of the pores are filled with water. The downward movement of water under the combined forces of gravity and film pressures is very rapid, as shown in figure 1 and also in figures 2 and 4 in which time is a factor. Although the distribution at the end of five days is surprisingly uniform, the final distribution condition requires a long period of adjustment.

DISCUSSION OF RESULTS

The experimental evidence indicates that when water is added to a body of uniform soil and allowed to percolate downward through the soil mass, a condition of distribution will be reached at which the moisture content at any depth will be approximately the same, except for a region of very rapid decrease in moisture present in the lowest five or six inches of wetted soil. This zone of rapid decrease has been designated the "taper."

The distribution above the taper, when graphically represented, closely approaches a straight line provided the sampling of the soil is delayed for a sufficient time to allow this condition of equilibrium to be reached. If the amount of water added be just sufficient to wet the soil down to a change in texture, the moisture distribution throughout the full depth will be uniform. If any additional water be added, the amount held at any level will be increased, but with larger amounts in the lower depths, until at the maximum moisture capacity the curve or gradient of moisture content will approach that of the upper section of a "capillary potential" gradient.

Plotting the distribution obtained by adding increasing amounts of water gives a "pendulum" swing to the curves. In figure 1, curves 2, 2½, and 3 show the "deficiency" curve with a slope to the left. A theoretical distribution of exactly the right amount of added water would be straight lines showing uniform distribution. Curve 3½ shows a slope to the right occurring when a slight excess of water was added. In figure 3 the curves representing distribution after application of 6, 7 and 8 inches of water show the same type of swing, the 6-inch curve having a "deficiency" slope to the left and the 8-inch curve an "excess" slope to the right. In figure 4 all the graphs show "deficiency" curves to the left.

The term "Normal Moisture Capacity" is proposed as an expression indicating the moisture content of the uniform distribution approximated in curve *D*, figure 4, showing the distribution after 123 days. This amount approaches closely the condition described by Widtsoe (9) as the "optimum Capillary Water Capacity or Field Water Capacity." However, the author's conception of the condition of this water is that it is essentially static, that it will not move

upwards, downwards, or laterally, a condition which Widtsoe recognized and designated as the "Lento-Capillary Point," but which he placed materially lower in amount of water than his "Field Water Capacity."

This water is held in the soil by two phenomena. A portion is taken up by the colloidal material present as absorbed or adsorbed water. This portion is very firmly held and is not free to move under any of the forces present in the soil. The second portion is the water film over the surface of the soil particles (and colloid coating) with waist-like accumulations where the particles are in contact. The force of gravity and the capillary forces directed downward will move the film water down until the angles of the surfaces at the points of contact are acute and sub-catenoid, giving them a high film pressure. The remaining film water can not then move in any direction in the soil, and would correspond to the condition of lento-capillarity.

If the surface were dried out, this moisture could not move upward to any measurable extent. The upper few inches might dry by direct evaporation from the surface and soil pores, and develop a "taper" comparable to that at the lower extremity of the wetted zone. The depth of this taper would depend on the texture and structure of the soil, the size of the soil particles, and the effective size of the pores of the soil. Upward movement to replace the moisture lost by such surface evaporation is exceedingly slight and the depth so affected is very limited; as the thinness of the films and the sharp angle of the contact surfaces are the result of the original "capillary" forces plus gravity, upward capillary or film movement against gravity will be negligible or non-existent. A slow movement of moisture may occur in the vapor phase by normal vapor pressure phenomena, which would cause a movement toward any region of drier soil, thus tending to equalize the moisture content throughout the soil column. A definite loss may occur through the movement of air in the pores of the soil, the daily out-movement as the soil is warmed and the air expands, and the corresponding in-movement at night when the soil cools producing a "ventilation" and a removal of water vapor that will slowly reduce the soil moisture. The amount thus lost is small and the rate of such loss decidedly slow. At the Normal Moisture Capacity, the soil moisture is considered to be in a static condition or at least in such a state that movement of the liquid phase in any direction is exceedingly limited and negligible.

WATER OF ADHESION

The term "Water of Adhesion" is proposed to designate the water held in the soils under these conditions, defined essentially as follows:

Water of Adhesion is that moisture in excess of the hygroscopic water, held in the soil by colloidal adsorption and as films surrounding the soil grains, not free to move under the normal influences of film forces.

The so-called "forms" of soil moisture would then become hygroscopic water, adhesion water, capillary water, and gravitational water.

Hygroscopic water is variously defined as the amount of water present in an air-dry soil, while the hygroscopic coefficient is defined by Hilgard as the amount of water a dry soil will absorb from a saturated atmosphere at a uniform temperature of 15°C.

Capillary water is usually designated as that water held on or between the soil grains by the adhesion between the soil and water and by surface tension forces and that is free to move under the influence of film forces.

Gravitational water is obvious—the excess water that can or will drain from a soil by gravity. It therefore should not properly enter into consideration as soil moisture but rather as a source of soil moisture.

Adhesion water would lie between the hygroscopic and the capillary water, covering the amount held between the hygroscopic coefficient and the point of lento-capillarity.

APPLICATION OF RESULTS

Irrigation. Applying water to the surface of a deep uniform soil would ultimately result in a moisture distribution at the Normal Moisture Capacity. Any additional water would serve to increase the depth of penetration, but not the amount held at any given depth, and if the amount added were sufficient to wet the soil beyond the reach of the roots, the excess moisture penetrating the lower layers would be lost to the plant. “Storing up” of water in a deep soil by winter irrigation or by the application of excessive amounts early in the season when water is abundant is impossible unless there is an impervious layer that will prevent further downward movement and result in a perched water table and a zone of saturation.

Mulches. When the soil is wetted to its Normal Moisture Capacity by rain or by irrigation, and this moisture is not free to move under the influence of film forces, as indicated in these experiments, then the “capillary rise” of this water is impossible and continued loss of moisture from the surface can not take place. The surface few inches will dry out to a depth about that of the usual mulch in arid regions. Beyond this drying can proceed only through vapor movements. A mulch can not prevent moisture movements which do not occur! Under such conditions therefore a mulch is of little or no effect.

Greenhouse practice. In experimental work in plant culture, soils are often moistened to the “optimum moisture content” before being placed in the pots, the seeds or plants are then planted and the system sealed. The loss of water by evaporation or transpiration, as determined by decreased weight, is replaced by adding the equivalent amount to the surface of the soil. Optimum Moisture Capacity is well below the normal Moisture Capacity. Water added to the surface will not be distributed uniformly through the soil mass until it reaches a uniform condition of “optimum,” but will remain at the “normal” moisture content, wetting a much shallower depth. The result is a wetted zone near the surface in which the roots can develop and grow, with

a lower zone dried down to the wilting point and more or less devoid of roots. This inequality in moisture distribution may vitiate the results of otherwise carefully controlled experiments.

CONCLUSIONS

A soil has a Normal Moisture Capacity which represents the minimum amount of water that is retained by absorption and film forces when the water is free to move downward through a mass of uniform soil.

The Normal Moisture Capacity is, for medium-textured soils, approximately the same as the moisture equivalent.

At the Normal Moisture Capacity the moisture is readily available for plants, but is not free to move under the normal film forces existing in the soil—it is essentially static.

In this condition, mulches are of little or no effect in conserving soil moisture, as upward movement to supply evaporation does not occur.

In deep uniform soil any water added by rain or irrigation penetrates the soil rapidly and soon approaches the Normal Moisture Capacity state of distribution.

REFERENCES

- (1) ALWAY, F. J., AND MCDOLE, G. R. 1917 The relation of movement of water in a soil to its hygroscopicity and initial moistness. *Jour. Agr. Res.* 10: 391-428.
- (2) BURR, W. W. 1914 The storage and use of soil moisture. *Nebr. Exp. Sta. Res. Bul.* 5.
- (3) BURR, W. W., AND RUSSELL, J. C. 1924 Report of certain investigations on the central Nebraska supplemental irrigation project. *Nebr. Dept. Pub. Works, 15th Ann. Rpt.* 1924: 199-240.
- (4) ISRAELSEN, O. W., AND WEST, F. L. 1922 Water-holding capacity of irrigated soils. *Utah Agr. Exp. Sta. Bul.* 183.
- (5) ROTMISTROV, V. G. 1913 The Nature of Drought; Odessa (Russia) Experiment Field, p. 1-48. Odessa, Russia.
- (6) SCOFIELD, C. S. 1924 The movement of water in irrigated soils. *Jour. Agr. Res.* 27: 617-695.
- (7) SHANTZ, H. L. 1925 Soil moisture in relation to plant growth. *Jour. Agron.* 17: 705-711.
- (8) VEHMEYER, F. J. Irrigation in relation to soil moisture and plant growth. Unpublished.
- (9) WIDTSON, J. W., AND McLAUGHLIN, W. W. 1912 The movement of water in irrigated soils. *Utah Agr. Exp. Sta. Bul.* 115.

THE HYDROMETER AS A NEW AND RAPID METHOD FOR DETERMINING THE COLLOIDAL CONTENT OF SOILS

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication December 10, 1926¹

Unquestionably one of the greatest needs in soil study is a very rapid and simple method for determining the colloidal material in soils, for the colloidal material seems now to constitute the seat of all the reactivities in soils. Such reactivities or relationships as base exchange, adsorbability and retainability of plant-food constituents, reactivity toward chemical reagents, solubility, heat of wetting, wilting coefficient, availability of water, evaporation of water, capillary movement of water, water holding capacity, rate of percolation, etc., are all controlled or influenced very largely by the colloidal content of a soil. In the last two years two methods which appear to come closest to giving a true estimation of the colloidal material in soils have been proposed. These two methods are the heat of wetting (1, 2) and the adsorption (4) methods. The chief drawback in both of these methods, however is that a portion of the colloids has to be extracted from the soil before the colloidal content can be determined, and this extraction process takes much time—in some soils, and where only small amounts are available it takes several days to extract enough colloids for an accurate adsorption or heat of wetting determination. Of the two methods, the heat of wetting is the quickest and simplest, but the adsorption method may take almost a week. It was once hoped that both the heat of wetting and the adsorption methods might be very rapid for estimating the colloidal content of soils, either the adsorption or the heat of wetting of the colloids being used as a factor, but it has been found that the colloids from different soils vary so greatly both in their adsorption and heat of wetting, that no factor can be used for all soils.

At this laboratory, therefore, attention and efforts have been directed elsewhere, with the hope of discovering a method or a factor which could be used for estimating very quickly and accurately the colloidal content of soils. This requirement seems to be happily furnished by the hydrometer method.

¹ At the request of the author this paper replaces that submitted July 23, 1926, "The Hydrometer as a New and Rapid Method for Estimating the Colloidal Material in Soils," a preliminary report of the investigations herein presented in final form. It is also given the position scheduled for "The Hydrometer as a New Method for the Mechanical Analysis of Soils" received for publication July 6, 1926, which thus relinquishes its priority rights as of July 6 and assumes those of July 23, 1926.

The employment of this method in connection with soil suspensions has revealed two remarkable facts; first, that the hydrometer can be used in measuring the rate of settling of soil particles (3) from which a distribution curve of the soil particles of various sizes might be worked out mathematically, and second, that it can estimate the colloidal contents of soils quite accurately and very rapidly.

It has been found that if a certain quantity of soil is dispersed in a definite amount of water, the percentage of the material that stays in suspension for 15 minutes, based on the original sample taken, is equal to the percentage of the colloids indicated by the heat of wetting method. This relationship is surprisingly close, with very few exceptions, and holds true for all types and horizons of soils and even when the quantity of soil is varied. The exceptions are mainly in soils that are unusually rich in organic matter especially in matter not thoroughly decomposed. Out of about 40 soils examined in the present investigation, however, which include a very good representation of all the different types and horizons, only 3 gave somewhat wide disagreement and this did not amount to more than 10 per cent.

On first consideration such a relationship as appears to exist between the percentage of colloidal material as determined by the heat of wetting method and the percentage of the material staying in suspension at the end of 15 minutes, might seem to be almost incredible or accidental. On further and deeper consideration, however, this relationship appears to be reasonable and logical. For instance, if different soils contain various amounts of fine or colloidal material, should we not expect a proportionate amount of fine or colloidal material to stay in suspension at a definite time, providing other factors did not interfere or intervene?

This rather remarkable relationship can be used now as a factor in determining the colloidal content of soils, which can be accomplished in 15 minutes. All that is necessary is to disperse the soil in a certain volume of water, determine the density at the end of 15 minutes, and from this density, which represents material in suspension, calculate the percentage of the sample in suspension which is the percentage of colloids that that particular soil contains.

The 15-minute period has been chosen because the relationship seems to be the closest for nearly all soils at this length of time. It was selected from hydrometer readings taken every minute or every few minutes, extending from 1 minute to 24 hours. The fact that this same time element holds true for nearly all soils is most remarkable and goes to show that there is a fundamental basis for the phenomenon.

The material that stays in suspension at the end of 15 minutes is probably not all colloidal, but the colloids that might be carried down or settle are apparently compensated by the non-colloidal material staying in suspension. The amount of non-colloidal material staying in suspension at the end of the 15-minute period, however, is probably very small.

THE HYDROMETER AND PROCEDURE

The hydrometer employed for measuring the quantity of fine material staying in suspension was of a special type made for this purpose, which had a very large volume and considerable weight, both of which tended to make it very sensitive and accurate. This hydrometer was calibrated so that it would indicate directly on its stem grams of solid material staying in suspension in one liter of water.

An essential part of the hydrometer method for determining the colloidal content is a complete dispersion of the soil. Four different methods were tried for this purpose. These included (a) the standard rocking shaker, (b) the beater used in beating eggs, (c) pestling in a mortar by hand, and (d) the drink mixer shown in plate 1. These different methods were able to disperse the same soil in different lengths of time. The rocking shaker required 6 hours to effect an approximately complete dispersion; the egg beater, 1 hour; the hand pestling, 15 minutes; and the drink mixer 3 hours in the original form, but as finally modified only 9 minutes.

It was desired that the method adopted for dispersing the soil be not only efficient and rapid, but also that it do away with the personal element. At the outset the drink mixer seemed to fulfill all the essential requirements.

The disappointment was very great, therefore, when it was discovered that in the form in which it is used for mixing drinks, more than three hours were required to effect a complete dispersion. Its failure to accomplish it in less time was due to the fact that the mixture in the cup was subjected to a circular motion without its being churned. Attention was directed, therefore, in correcting this defect, and happily it was most successfully corrected by putting a series of copper wires on the wall of the container to act as obstructions, and to prevent the circular motion.

The difference this slight change in the walls of the container made on the efficiency of the dispersing ability of the machine, is most remarkable, as seen by the fact that the time was reduced from more than 3 hours to only 9 minutes.

This machine in its final form, now, is a most effective machine for the dispersion of the soils.² The motor, without a load, goes at a speed of about 14,000 revolutions per minute and with a load, at about 9,000 revolutions per minute. The energy expended in dispersing the soil, therefore, can be easily imagined.

The method of pestling by hand in a mortar is very efficient, and very rapid,

² The idea of using this machine in the original form, was suggested in a conversation by Prof. H. Ries of Cornell University. As already stated, however, in the original form this machine is not practical for this work.

This machine is manufactured by the Hamilton Beach Manufacturing Company of Racine, Wisconsin, who have undertaken to make the special cups to be used in dispersing the soil. The complete outfit costs about \$20.00

but the results are not always uniform and the dispersions not always as complete as that yielded by the motor and consequently is not recommended. With hand the soils can be dispersed in from 10 to 20 minutes, but they must be pestled vigorously even though the particles seem to be ground. They must be pestled and washed until all the soil is dispersed. The washings must stand half a minute before being decanted into the cylinder.

The length of time required for soil dispersal, at which the results of the hydrometer method correspond the closest with the results of the heat of wetting method, is 9 minutes for all the soils except for those whose organic matter content is either not well decomposed, or is difficult to disperse. Many soils with high contents of organic matter, from Illinois, Iowa, Michigan, and Minnesota behaved like the mineral soils with no organic matter, but a few soils, such as the Minnesota Carrington silt loam would not be dispersed at 9 minutes, but required about 30 minutes. Out of 15 of such organic soils examined, only 2 failed to give very close agreement with the heat of wetting method when dispersed only for 9 minutes. In such soils the organic matter could readily be seen to be not well decomposed. Such soils, which are readily recognized, should be stirred 30 minutes; all the other soils with organic matter should be stirred only 9 minutes. Those that need to be stirred 30 minutes will be exceedingly few according to this investigation thus far conducted. On the other hand, the error that might arise if these exceptional soils are stirred only 9 minutes, may not amount to more than 10 to 15 per cent. In the case of mucks and peats the method is not very successful because it is almost impossible to disperse those organic materials.

The 9-minute period of dispersion seems to effect almost a complete dispersion. For instance, Ontonagon silt loam gives 62.37 per cent of colloids when stirred 9 minutes and 66.20 per cent when stirred for 3 hours. Stalwart sandy loam gives 28.46 per cent when stirred only 9 minutes and 31.72 per cent when stirred 3 hours. These differences are certainly small when the great difference in time of stirring between 10 minutes and 180 minutes is taken into consideration.

In order to facilitate the dispersion, keep the material dispersed, and prevent flocculation and thus settling according to its physical laws, to each soil sample was added 5 cc. *N* KOH during the dispersion process. Most of the soils do not need the addition of KOH, because they are easily dispersed and remain suspended according to the physical laws governing the size of their particles, but there are some soils which have a tendency to flocculate after they are dispersed, and tend to settle in mass when they begin to flocculate. The addition of the KOH prevents this tendency to flocculate and the soil particles settle in the regular manner. In order that the general procedure might be uniform, the KOH was added to all the soils. This addition increased very little, if any, the amount of material that stayed in suspension at the end of 15 minutes, except in those soils that have a tendency to flocculate. Apparently the KOH serves the purpose mainly of stabilizing the soil

particles or colloids which have a tendency to flocculate. Some soils require more KOH than others in order to be stabilized.

The general procedure in the actual determination consisted of weighing out an amount of soil equivalent to 50 gm. on the oven-dry basis, although for practical purposes the hygroscopic moisture can be estimated if air-dry soils are used and allowance is made in weighing out the samples, placing it in the cup and filling the latter with distilled water to within 1 inch of the top. The cup was then connected to the stirrer of the motor and was stirred for exactly 9 minutes. The mixture was poured and washed into a liter cylinder having a total capacity of 1250 cc., a diameter of $2\frac{1}{2}$ inches, and a height of 18 inches. The hydrometer was then placed in the mixture and the cylinder containing the hydrometer filled to the top with distilled water. The hydrometer was taken out and the suspension was vigorously shaken by hand with the palm of one hand acting as a stopper. The cylinder was then placed on the table and the time immediately noted by a stop watch. The hydrometer was then placed in the mixture and at the end of 15 minutes the reading of the liquid was taken. Just half a minute before the end of 15-minute period, the hydrometer was gently pushed down into the liquid, to avoid any error in the reading caused by lag or sticking of the hydrometer. The temperature of the mixture was also noted. The amount of material shown on the hydrometer was then divided by 50, which was the amount of sample taken, and the result was the percentage of colloids in that soil.

In taking the sample, care should be exercised that the heavy types of soil are not in big lumps, because the water requires longer time to penetrate and to disperse these lumps. Also, soils that have a heavy content of alkali salts, should be washed before using. The samples taken should also be a very good representative of the whole soil.

With the 50 gm. of soil 1050 cc. of water was used. In the calculation of the results, however, only 1000 cc. was employed; the other 50 cc. was considered to be used up in wetting or saturating the soil.

In order to test out the method, various quantities of soil such as 25, 50, 75 and 100 gm., were employed. In every case 1000 cc. was used and in addition 1 cc. for every gram of soil, to make allowance for the water required to wet or saturate the soil. This additional water was added because the hydrometer measures the amount of suspensions in the entire column of liquid irrespective of the density with depth. In other words the hydrometer readings give the average of the densities for the entire column of liquid, i.e. from the top to the point where the solid column of soil is being formed by settling. Hence, to make the liquid columns above the various soils comparable, allowance was made for the water required to saturate the soils.

The hydrometer can be best read, in some cases when the column of liquid is at the top of the cylinder. In order, therefore, to have 1050 cc. of water with 50 gm. of soil and at the same time to have the column of the liquid at the top of the cylinder with the hydrometer floating in the liquid, cylinders of the

required size have been made.³ With the use of these special cylinders, the results are comparable, matters are simplified, and time is saved in not having to measure the water. One can use, however, any regular liter cylinder that has a total capacity of about 1250 cc. An ordinary 1000 cc. cylinder may also be used by making a mark of the proper volume.

Changes of temperature make a difference in the results. A change of 1°F. makes a difference of about 0.350 per cent of soil colloids. For absolute comparison, therefore, the results must be reduced to the same temperature basis. In the present investigation the temperature of 67°F. has been chosen as a basis which is also the temperature at which the hydrometer has been calibrated. For readings above 67°F., therefore, 0.35 per cent must be added for each degree, to the percentage of colloids indicated by the hydrometer; and for readings below 67°F., 0.35 per cent must be subtracted for every degree of temperature. An example will make this point clear. Suppose that a soil gave 60 per cent of colloids at the temperature 77°F. To this 60 per cent must be added 3.5 per cent which will make a total of 63.50 per cent for this soil at the temperature of 67°F. If on the other hand this soil gave 67 per cent of colloids at the temperature of 57°F., from this amount must be subtracted 3.5 per cent.

The hydrometer was constructed to have a range between 0 and 60 gm. of dry material per liter of water and, therefore, can measure the colloidal content of a soil which may be as low as 1 per cent, taking a 50 gm. sample or 0.5 per cent taking a 100-gm. sample. It is graduated in 1 degree. This wide range of readings on the stem of the hydrometer was not needed in the colloidal determination but was placed on the hydrometer so that the latter could be used also in studying the rate of settling of soil particles, from which a distribution curve could be worked out showing the sizes and amounts of the soil particles. As shown in another publication (3) the hydrometer method lends itself in a remarkable way to this type of study. The hydrometer can be made considerably more accurate and sensitive by reducing its scale.

Many factors, such as flocculation, which might influence the rate of settling of soil particles on long standing are not effectively operative in short periods. Even those soils which have a tendency to flocculate are prevented from doing so by the addition of small amounts of KOH.

With only 9 minutes required to disperse the soil and only 15 minutes needed to make a hydrometer reading, the colloidal content of soils can be determined very quickly. Considering that after the first soil is dispersed, the second soil can be dispersed while the hydrometer readings of the first soil are being made, the colloidal content of more than three soils can be determined in one hour with one hydrometer.

Probably there are very few, if any, methods in soil physics work which are standardized so well as this method for determining the colloidal content of

³ The cylinders are handled by the Taylor Instrument Company, Rochester, N. Y., who are also handling the hydrometers, and cost about \$1.50 apiece.

soils. The hydrometer, for instance, floats and is governed entirely by physical laws with no outside disturbing factor and no personal element entering into it. The soil is dispersed at a definite speed and at a definite time by a mechanical means.

The method may appear arbitrary, but it is quite absolute because it is based upon the heat of wetting method which is quite absolute as far as it goes. Parker and Pate (5) made a comparison of the heat of wetting method with the water adsorption and base exchange method and found it to agree very well with these two methods and especially with the base exchange method. The procedure is arbitrary, but the results are absolute. Furthermore, considering that colloids are fundamentally a question of size of particles, and that the hydrometer method measures, in a final analysis, the size of particles, it seems to be the logical method for such a determination.

By following the general procedure outlined above the results of any soil can be duplicated almost exactly any number of times, provided that the temperature is constant or correction is made for it and also provided that a representative sample of the soil is taken every time.

Referring once more to the dispersing machine, there are two things that must be strictly guarded against: first, the cup must have the baffles or wires in it; and second, the paddle or button on the stirring rod tends to wear out on sandy soils; when it becomes flat, it must be replaced because in the flat condition it loses its stirring efficiency. With these two precautions, it can be said that this machine is most wonderful for dispersing soils for any purpose.

EXPERIMENTAL DATA

In table 1 are presented the data showing the relationship between the percentage of colloids as obtained by the heat of wetting method, and the percentage of the material staying in suspension in a liter of water at the end of 15 minutes, based on the amount of soil sample taken. There were four different quantities of soil employed 25, 50, 75 and 100 gm. per liter of water plus the additional water required for saturating the soil, but in this table the results for only the last three quantities are shown. The soils employed or tested include almost all the different classes coming from different localities and horizons. Many of these soils contain also various quantities of organic matter. These soils would certainly seem to represent an excellent array of diverse and representative types of soils for testing out the method.

An examination of table 1 shows at once that the relationship between the percentage of the colloids as determined by the heat of wetting method and the percentage of the material staying in suspension in a liter of water at the end of 15 minutes is very close in the majority of the soils. In many of the soils it is almost exactly the same, in a few it is not so close. Out of the 39 soils shown in the table, 3 show the widest disagreement. These are Strongs B₁ sandy loam, Minnesota Carrington silt loam, and California Yolo loam.

TABLE 1

Relationship between the percentage of colloids as indicated by the heat of wetting method and the percentage of material staying in suspension at the end of 15 minutes in a liter of water based on the weight of sample taken. Soils dispersed 9 minutes and results reduced to 67°F.

SOILS	PROPORTION OF COLLOIDS BY HEAT OF WETTING	PROPORTION OF COLLOIDS BY HYDROMETER METHOD		
		50-gm. samples	75-gm. samples	100-gm. samples
	per cent	per cent	per cent	per cent
Hillsdale sandy loam A	15 73	18.35	18 86	17 50
Hillsdale sandy loam B ₁	18 70	19 00	19 25	18 60
Hillsdale sandy loam B ₂	20 42	18 32	17 00	17.61
Strong's sandy loam A ₁	5 18	8 30	8 48	8.74
Strong's sandy loam B ₁	23 12	14 23*	13 85	14 10*
Chippewa fine sandy loam A ₂	12 02	11 68	10 32	10.82
Fox loam A ₂	20.21	23 32	22 01	23.80
Fox loam B ₁	27.65	27.00	28 30	28.47
Fox loam B ₂	20 58	18 08	19 12	18.22
Onaway loam A ₁	11 66	11 69	12 18	13 07
Onaway loam B ₁	21 03	20 65	20 08	19 65
Onaway loam C	28.44	29 52	27 83	28 70
Stalwart loam A ₁	22 49	24 22	24 02	25 10
Stalwart loam A ₂	25 61	28.46	27 30	28 15
Stalwart loam B ₁	21 01	24 87	25 10	26.00
Napaneer silt loam A ₁	35.51	39 98	40 38	40.83
Napaneer silt loam B ₁	41 09	48 10	47 60	46.90
Napaneer silt loam C	50 76	53 08	52 10	51.65
Ontonagon silt loam A ₁	60.65	62 57	62 00	61.54
Ontonagon silt loam A ₂	60 30	63 82	62 90	62 08
Ontonagon silt loam B ₁	71 30	73 06	72 80	71.80
Pennsylvania silt loam	24.50	29 88	26 10	25 70
Ohio silt loam	31 20	36 42	36 12	37.10
Tennessee silt loam	31.41	34 62	33.74	34.51
Michigan silt loam	39 04	44 60	44.18	43.10
Illinois clay loam	34.52	37.20	36.80	36.30
California yolo loam	48.51	38 00*	38.62*	37.40*
California yolo clay loam	59.54	53 94	54 10	53 65
California yolo clay	58 34	56.80	56 03	55.94
Minnesota Marion silt loam	45 40	36 95	37 18	37.62
Minnesota Carrington silt loam	50 62	38.12*	37.85*	37.02*
Iowa Bremer clay	49 40	51.69	51.03	50.90
Calcareous clay C	93.20	86.20	87.19	86.65
Brookston clay loam A	33.40	32.72	33 80	32.92
Brookston clay loam A ₂	36.10	38 90	39.10	38.60
Brookston clay loam B	38.62	39.00	38.40	38.10
Greenville clay loam B	52 06	48 85	47 72	48 08
Cecil loam clay loam B	58.03	60.70	60.10	59.55
Manor schist loam B	34 00	30.01	30.90	30.60

* Not dispersed in 9 minutes.

The Strong's sandy loam was reddish brown and seemed to contain organic matter mixed with iron, which was hard to disperse in 9 minutes. The Minnesota Carrington silt loam contained considerable organic matter probably not in a thoroughly decomposed state and would not disperse in 9 minutes, but did in about 30 minutes. Other soils containing as large as, or larger organic matter than, the Carrington silt loam, such as the Illinois clay loam, Iowa Bremer clay, Michigan Brookston clay loam, and others, were easily dispersed in 9 minutes and as will be seen, gave a very good relationship. The California Yolo loam does not give such a big disagreement, but it does not give so close agreement as the other two soils of its series, Yolo clay loam and Yolo clay. No possible explanation can be suggested for this disagreement unless this soil contained some colloidal material which was not completely dispersed in 9 minutes.

The disagreement in the three soils mentioned above, although not very great, probably serves the purpose of indicating that there may be exceptional and abnormal soils which may not follow the relationship very closely. Excepting these unusual cases, however, the relationship is remarkably close considering the diversity of the soils, and the difficulty of taking absolute representative samples of soils for both sets of experiments. The hydrometer method would seem to be justified, therefore, in being adopted to determine the colloidal content of soils in 15 minutes as compared to one week, which is required by our present methods. It might be added here also that the agreement in the results between the heat of wetting and hydrometer methods is a great deal closer than the agreement in the results between the dye, water vapor, and ammonia methods as obtained by Gile et al. (4).

It is of the greatest significance to note that whether 50, 75, or 100 gm. of soil was employed, the percentage of colloids is the same in the different quantities and agrees with the percentage of colloids as determined by the heat of wetting method. This fact is very important, for it goes to show that the principle of the hydrometer method is basic and sound, and that the relationship between the two methods is fundamental and not accidental.

A very interesting thing revealed by the foregoing results is the fact that even soils with considerable amounts of calcium carbonate give a very good relationship. Calcareous clay and most of the soils from the C horizon contain considerable amounts of calcium carbonate and yet they show close relationship.

Since the hydrometer when floating follows entirely fundamental physical laws and is not disturbed by outside factors, the amount of material it indicates in a liter of water at the end of 15 minutes or at any length of time for the different soils, must be of the same average size of particles in the different soils. In other words, when two soils show different amounts of material in suspension in a liter of water at the end of a given time, this suspended material must be of the same average size of particles in both soils. Here then, is another quick way of determining the amount of material of the same average

size of particles in the different soils. It must be remembered that everything being equal, the rate at which soil particles settle, is governed mainly by their size.

Since the hydrometer method gives absolutely comparable results for the different soils, and since these results show such a remarkably close relationship with those obtained by the heat of wetting method, it goes to prove that the heat of wetting method has been a correct method for determining the colloidal content of soils. The hydrometer method apparently tends to measure the same thing as the heat of wetting method.

Following the above facts and reasoning further, it would seem reasonable to expect that if there were no experimental error in the heat of wetting method and if the soils were all completely dispersed in the hydrometer method, the results of the two methods would be still closer than they are.

The hydrometer method lends itself to determining the colloidal content of a soil in any condition of moisture, that is, whether the soil is oven-dry, or moist. If there has been a belief, therefore, that drying the soils affects their colloidal content, it can be easily tested out by this method. Preliminary studies with this method indicate, however, that a dried soil gives practically the same colloidal content as before drying. This statement, however, may not apply to all the organic soils.

Although all the different quantities of soil in the same quantity of water that have been employed, showed the same percentage of material in suspension at the end of 15 minutes, 50 gm. is probably preferable, except in soils which have a very low colloidal content, where 100 gm. is preferable.

In view of the remarkably consistent closeness of the relationship in many diverse types of soils between the percentage of colloids as shown by the heat of wetting and the percentage of the material staying in suspension at the end of 15 minutes in a liter of water, and in view of the fundamental basis governing this relationship, it would seem only logical to employ this relationship as a factor for determining very rapidly the colloidal content of soils.

Finally, by referring once more to the foregoing experimental results, it will be seen readily that the colloidal content of soils is infinitely higher than formerly was thought. This is, however, a new concept that is being forced upon us by the newer methods of studying colloids. These new methods are the water adsorption (4), base exchange (5), heat of wetting (2), and the hydrometer method. All these methods seem to agree pretty well in indicating this high colloidal content of soils.

If this high colloidal content is correct, it means that our present textural classification of soils is wrong and has to be changed radically to conform to these new concepts. Investigations looking toward a textural reclassification of soils are being conducted at this laboratory.

SUMMARY

The hydrometer method is presented as a new and very rapid method of estimating the colloidal content of soils.

By means of this method the colloidal content of a soil can be determined in 15 minutes. The colloidal content of three soils can be determined in less than one hour.

This is accomplished by the aid of a factor which has been discovered to exist between the colloidal content of a soil as determined by the heat of wetting and the percentage of the material staying in suspension in a liter of water at the end of 15 minutes. In other words, the percentage of the material staying in a suspension at the end of 15 minutes, as indicated by the hydrometer method, is equal to the percentage of colloids as determined by the heat of wetting method. There seems to be a fundamental basis for this relationship, as it has been found to hold remarkably close in all classes and types of soils, with very few exceptions which are due to rather undecomposed organic matter, or organic matter very difficult to disperse.

The soils are dispersed by a stirrer driven by a very fast motor and are contained in a specially constructed cup. The length of dispersion is 9 minutes except in a few special cases. To each soil sample is added 5 cc. of *N* KOH to stabilize the soil particle or colloids, as some of the soils have a tendency to flocculate. The hydrometer when floating is governed entirely by absolute physical laws. That being the case, the results it gives for the different soils must be absolutely comparable. This means then that the amount of soil material staying in suspension at any given time in a liter of water must be of the same average size of particles for the different soils since, everything being equal, the rate at which soil particles settle, is governed by their size.

Since the hydrometer method gives absolutely comparable results for the different soils and since these results show a very close relationship with the results of the heat of wetting method it means then that the heat of wetting method has been a correct method for determining the colloidal content of soils. Evidently both methods tend to measure the same thing.

By following the directions closely for executing the determination, the results of any soil can be duplicated almost exactly any number of times.

A new and special hydrometer has been constructed for measuring the density of the soil suspensions, which is very accurate and sensitive and gives grams of dry material per liter of water directly.

It is believed that this hydrometer method can be employed now with confidence and assurance for determining the colloidal content of soils and such determination seems to be accurate, except in a very few special cases.

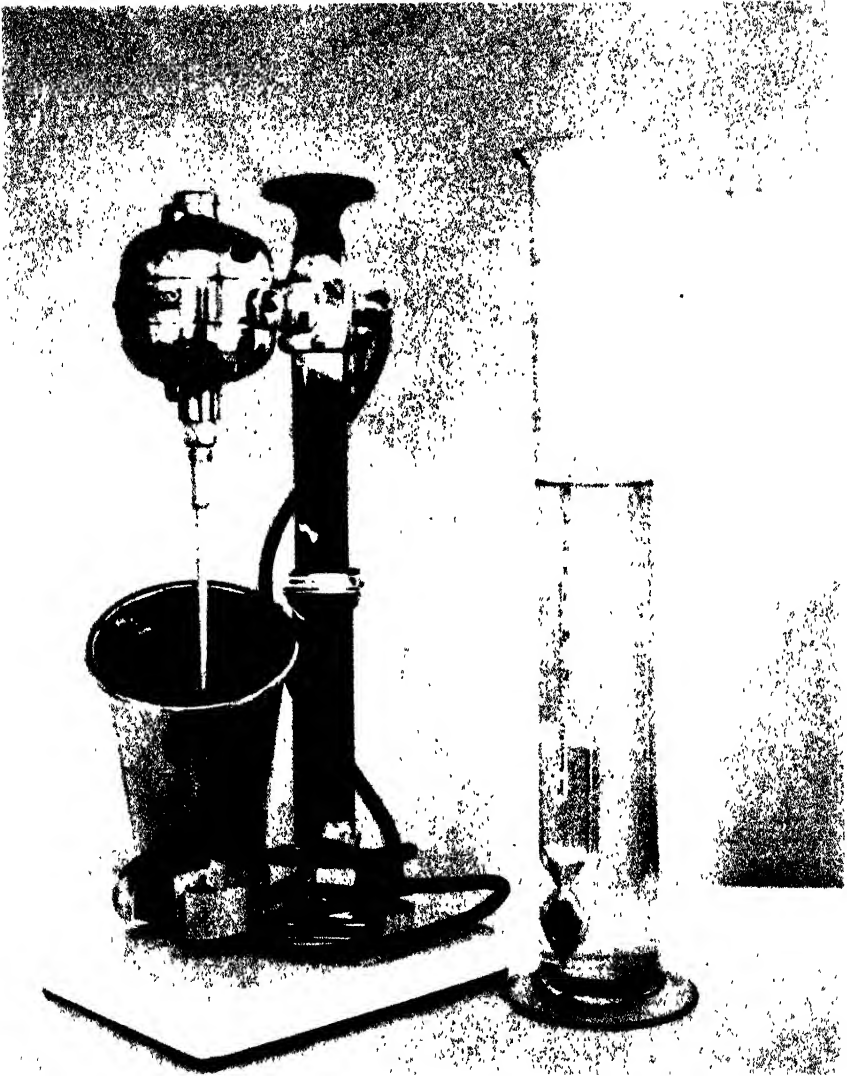
Since the colloidal content of soils, as indicated by the hydrometer method, as well as by the heat of wetting, water adsorption, and base exchange methods is infinitely higher than used to be believed, a new and radical textural reclassification of soils is needed.

REFERENCES

- (1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.*
- (2) BOUYOUCOS, G. J. 1925 Heat of wetting as a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153-162.
- (3) BOUYOUCOS, G. J. 1925 The hydrometer as a new method for the mechanical analysis of soils. *Soil Sci.*—in press.
- (4) GILE, P. L., et al. 1924 Estimation of colloidal material in soil by adsorption. U. S. Dept. Agr. Bur. Soils Bul. 1193.
- (5) PARKER, F. W., AND PATE, W. W. 1926 Base exchange in soil colloids and the availability of exchangeable calcium in different soils. *Jour. Amer. Soc. Agron.* 18: 470.

PLATE 1

SHOWING THE DISPERSING MACHINE, CYLINDER AND HYDROMETER, IN THE DETERMINATION OF SOIL COLLOIDS



BOOK REVIEW¹

Données Numériques de Cristallographie et de Minéralogie. By M. L. SPENCER. Section D of *Tables Annuelles de Constantes et Données Numériques de Chimie, de Physique et de Technologie.* Edited by Ch. Marie. Vol. V—Years 1917 to 1922, inclusive: Part 1, pp. i-xl, 1-804 (1925); Part 2, pp. i-iii, 805-1934 (1926). Gauthier-Villars & Cie, Paris; The Cambridge University Press, Cambridge; University of Chicago Press, Chicago, 1925. Price, cloth, \$25.00, paper \$22.00.

Section D, Crystallography and Mineralogy (pages 1285-1403) comprises six subdivisions (in French), as follows: I. Crystallography of Minerals, II. New Crystalline Form of Minerals, III. Crystallography of Inorganic Compounds, IV. Crystallography of Organic Compounds, V. Structure of Crystals from X-ray Data, and VI. Miscellaneous Data. (Elements in earth's crust, specific heats of various minerals, hardness of minerals and rocks, and so forth.)

Volume V of the Annual Tables, of which Section D is a part, contains data accumulated during the years 1917 to 1922 inclusive. The publication of a volume each year was interrupted by the World War and the inclusion of six year's data in one volume has been necessitated by the afterwar conditions in many countries.

These Tables are not intended to take the place of, or compete with, the International Critical Tables. They are planned to make available an annual non-critical summary of the material obtained during a current year, it being the function of the Critical Tables to present the data after a critical examination.

The present volume is in seven sections. It may be purchased in sections or as the complete volume.

¹This review was prepared by Prof. Walter C. Russell, of the New Jersey Agricultural Experiment Station.

EFFECT OF FERTILIZERS ON THE GERMINATION OF SEEDS¹

JACOB L. MAXTON

Virginia Polytechnic Institute

Received for publication July 10, 1926

It is becoming an increasingly widespread practice in some sections for farmers to mix the seeds of grasses and clovers with fertilizers and to sow the seed and fertilizers together. The question is frequently asked whether the seed will be injured if they are not sown immediately after they are mixed with the fertilizer. The investigation reported in this paper was started to answer this question.

MATERIALS AND METHODS

The seeds used were alsike clover, Canada bluegrass, creeping bent grass, crimson clover, Kentucky bluegrass, lespedeza, meadow fescue, orchard grass, redtop, red clover, sapling clover, sheep's fescue, tall meadow oat grass, timothy, white clover, white sweet clover. All the different kinds of seeds showed good germination between moist blotting paper in the laboratory. Two hundred seeds of each kind were placed in contact with various fertilizers for one-week, two-week, three-week, and four-week periods. The fertilizers used were acid phosphate (16 per cent P_2O_5), ammonium chloride, ammonium nitrate, ammonium phosphate, ammonium sulfate, calcined phosphate, calcium carbonate, calcium cyanamide, calcium oxide, potassium chloride, potassium sulfate, and synthetic urea. The seeds were planted in Hagerstown silt loam in the greenhouse. The soil was held as far as practicable at optimum moisture conditions. The rates of application of the fertilizers are shown in table 1.

RESULTS

Germination as affected by fertilizers

As shown by the data in table 2 calcium cyanamide was most injurious to germination. However, all of the fertilizers reduced germination to some extent. In some instances urea reduced germination to a marked degree.

The different kinds of seeds were affected in different degrees by the fertilizers. Meadow fescue, with the exceptions of calcium cyanamide and calcium carbonate treatments, germinated higher when in contact with fertilizers than when seeded without being in contact with fertilizers. Timothy seed

¹ Abstract from minor thesis in agronomy submitted in partial fulfillment of the requirements for the degree of Master of Science.

apparently was injured but little by being seeded in contact with different fertilizers, except calcium cyanamide. Kentucky bluegrass, and white sweet clover seeds were injured more by the fertilizers than the other seeds used, as shown in table 3.

The average percentage germination of grass seeds under all treatments for all the periods was 70, as compared with 100 for the checks, and with 62 to 100 for the other kinds of seeds. In other words, there was practically no difference in the reduction in germination of seeds of grasses and those of the other kinds used.

Effect of time of contact of seed with fertilizer on the germination

The results shown in tables 2 and 3 indicate that the injury to germination is not affected by the seeds being in contact with the fertilizers different lengths

TABLE 1
Rates of applying fertilizers per acre

FERTILIZER	AMOUNTS
	<i>pounds</i>
Acid phosphate	200
Ammonium chloride.....	100
Ammonium nitrate.....	100
Ammonium phosphate.....	100
Ammonium sulfate.....	100
Calcined phosphate.....	200
Calcium carbonate.....	500
Calcium cyanamide.....	100
Calcium oxide.....	250
Potassium chloride.....	100
Potassium sulfate.....	100
Urea.....	50

of time before being sown. There are no consistent differences between the effects on germination with the seeds remaining in contact with the fertilizers for 1, 2, 3, or 4 weeks. The length of time seeds are in contact with dry fertilizers is inconsequential.

The data in table 2 do not show whether the injury occurred before or after the seeds were planted. In order to study this point further, the seeds of crimson clover and tall meadow oat grass were placed in contact with the fertilizers for 4 weeks. Some of the seeds were then separated from the fertilizers and seeded and some were left in contact with the fertilizers and seeded. The results as shown in table 4 indicate that all the injury was produced after the seeds were planted and that the contact of the seeds with the dry fertilizers for the 4-week period did not injure the germination.

The ability of the soil to counteract the injurious effects of fertilizers on germination of crimson clover was determined by applying the fertilizers in

TABLE 2

Percentage of germination of clovers and grasses when placed in contact with different fertilizers for varying periods of time and then placed in the soil with the fertilizers

SEEDS	Lespedeza				White clover				Kentucky bluegrass				Canada bluegrass				Meadow fescue			
Germination	per cent				per cent				per cent				per cent				per cent			
Weeks of contact	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<i>Treatment</i>																				
Check.....	70	60	59	53	42	37	66	52	22	18	28	24	20	44	36	34	20	29	28	26
Ammonium sulfate.....	54	44	57	57	16	29	29	58	14	14	12	8	28	8	10	26	30	33	29	27
Acid phosphate.....	61	48	67	51	55	38	33	47	8	8	4	4	14	14	22	14	28	29	42	34
Calcium oxide.....	42	34	57	21	24	32	24	24	8	8	8	10	18	16	8	20	27	26	16	31
Ammonium chloride	21	36	30	35	33	31	29	59	6	6	14	6	18	6	4	28	25	28	30	26
Check.....	68	82	83	58	50	33	59	55	22	28	24	20	24	20	36	30	22	26	22	22
Calcined phosphate.....	56	36	45	54	23	31	66	40	16	22	20	10	20	26	28	30	26	30	30	23
Potassium sulfate.....	26	56	62	38	16	34	50	26	2	4	8	6	20	22	28	18	29	33	30	30
Ammonium nitrate.....	23	40	45	32	19	16	48	32	8	20	12	6	14	16	10	22	27	32	25	22
Potassium chloride.....	28	34	51	38	26	28	35	28	9	16	4	6	8	10	12	12	29	32	36	28
Ammonium phosphate.....	46	47	47	58	4	9	27	28	8	6	6	6	8	12	12	24	22	36	27	34
Check.....	50	72	71	59	44	54	59	41	18	24	14	20	42	28	18	16	17	22	29	27
Urea.....	52	62	50	62	18	10	29	23	9	10	4	8	10	20	22	10	24	18	22	27
Calcium cyanamide.....	8	5	6	14	10	2	11	7	2	0	0	2	0	2	0	0	0	0	0	0
Calcium carbonate.....	62	48	61	40	30	24	55	42	26	26	34	16	30	32	22	32	27	19	20	14

SEEDS	Sheep's fescue				Red clover				Alsike clover				Sapling clover				Crimson clover				White sweet clover			
Germination	per cent				per cent				per cent				per cent				per cent				per cent			
Weeks of contact	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<i>Treatment</i>																								
Check.....	18	18	20	18	38	39	42	45	61	50	48	45	35	41	35	38	29	23	20	21	24	22	20	19
Ammonium sulfate.....	20	24	28	17	23	51	42	50	44	35	22	47	17	18	18	10	12	17	18	11	15	6	5	8
Acid phosphate.....	14	11	6	3	30	24	24	50	39	53	27	61	28	39	31	28	21	15	16	9	15	14	12	14
Calcium oxide.....	27	19	31	20	28	35	31	25	32	43	42	31	35	32	49	43	14	12	14	13	20	14	16	19
Ammonium chloride	23	12	12	14	34	22	22	20	35	49	28	45	14	11	7	4	18	15	6	18	9	6	6	7
Check.....	22	27	19	21	50	55	60	61	59	65	67	60	33	45	56	49	28	25	21	17	23	22	14	24
Calcined phosphate.....	15	8	23	29	54	42	54	55	54	54	56	55	41	36	36	40	27	22	21	14	22	21	12	10
Potassium sulfate.....	22	20	20	13	64	33	50	59	45	58	61	60	30	26	26	14	26	16	15	14	18	15	9	7
Ammonium nitrate.....	15	18	17	12	42	29	22	39	55	60	52	28	14	22	11	4	14	15	21	13	7	6	5	4
Potassium chloride.....	24	25	25	18	48	33	42	53	45	40	54	54	28	25	20	16	48	28	29	32	20	12	9	10
Ammonium phosphate.....	25	21	14	6	28	20	27	25	18	38	38	37	12	19	12	8	22	8	9	12	5	7	5	3
Check.....	19	22	20	19	65	63	66	59	51	52	69	61	40	36	47	42	31	20	19	18	25	20	18	21
Urea.....	25	19	9	11	8	10	6	10	10	14	25	16	19	27	18	13	14	8	5	12	5	4	3	2
Calcium cyanamide....	0	0	0	0	3	3	1	2	5	6	4	7	3	2	2	2	4	1	2	2	3	2	4	2
Calcium carbonate.....	20	33	31	20	51	61	50	61	67	59	48	39	42	36	53	34	28	14	27	14	20	19	19	18

TABLE 2—*Continued*

SEEDS	Tall meadow oat grass				Rhode Island bent grass				Orchard grass				Creeping bent grass				Timothy			
Germination	<i>per cent</i>				<i>per cent</i>				<i>per cent</i>				<i>per cent</i>				<i>per cent</i>			
Weeks of contact	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<i>Treatment</i>																				
Check.	52	43	36	43	12	9	10	14	20	32	22	28	38	42	29	51	92	90	86	88
Ammonium sulfate.	50	49	49	43	3	11	8	7	8	16	8	6	23	36	31	34	81	74	74	72
Acid phosphate	49	51	34	38	8	9	11	12	16	20	20	18	14	38	30	39	84	90	86	84
Calcium oxide.	37	35	44	41	2	0	3	4	24	16	24	24	31	30	40	34	74	72	70	67
Ammonium chloride.	40	32	44	35	7	9	9	8	24	10	16	22	18	33	34	36	58	74	74	70
Check.	44	44	49	44	9	17	11	14	24	22	28	30	50	44	56	46	84	83	88	84
Calcined phosphate.	36	33	33	31	4	6	5	6	28	28	24	36	34	48	33	55	91	90	90	86
Potassium sulfate.	38	40	34	31	3	7	9	6	10	20	16	18	30	25	43	32	36	78	71	82
Ammonium nitrate.	46	66	37	36	7	6	8	5	22	18	22	18	10	22	28	34	26	70	72	56
Potassium chloride.	45	39	51	40	10	8	10	12	14	18	22	10	13	21	31	51	38	80	82	70
Ammonium phosphate.	45	42	56	42	7	8	11	9	24	24	16	14	8	18	14	24	48	82	68	72
Check.	48	46	38	46	9	17	8	14	24	22	24	30	38	54	68	60	86	90	82	80
Urea	26	27	33	22	11	14	9	5	14	12	6	4	8	9	23	9	77	44	52	44
Calcium cyanamide.	0	0	1	3	2	0	1	0	4	2	6	4	2	0	3	0	1	0	2	2
Calcium carbonate.	45	39	35	30	4	7	9	8	18	16	20	18	49	52	62	64	88	91	90	91

the row and allowing a 5-day period to elapse before sowing the seed. The results presented in table 4 indicate that by applying fertilizers several days before sowing the seeds, the harmful effects of fertilizers on germination can be largely overcome.

Nature of the injury to the germination of seeds by fertilizers

The question frequently arises whether the injury resulting from applying fertilizers in contact with seeds takes place before or after the radicles or plumules emerge from the seed coats. Sherwin (2) found that germination was inhibited by inorganic fertilizers and the seedlings injured by organic fertilizers while Hicks (1) and Shive (3), using inorganic fertilizers, found that injury occurred to the seedling.

The seeds of crimson clover and timothy were planted in contact with 500 mgm. of various fertilizers in 50 gm. of sand in Petri dishes and the sand was held at 50 per cent of its saturation. The fertilizers used were ammonium sulfate, calcium cyanamide, potassium sulfate, and urea.

The seeds were left in the sand for 10 days. Some of them swelled, but no germination took place. The harmful effects of the fertilizers in these cases evidently were due to the injury to the seed before germination rather than to the seedling.

From the experiments conducted to study the effect of solutions of the fertilizer salts of different concentrations on the plasmolysis of the seedlings and the

TABLE 3

Percentage of germination of clovers and grasses when placed in contact with different fertilizers for varying periods of time and then placed in the soil with the fertilizers

Average for all treatments

SEEDS	TREATMENT	GERMINATION OF SEED TREATED				GERMINATION FOR ALL PERIODS OF CONTACT AS COMPARED WITH CHECK
		1 week	2 weeks	3 weeks	4 weeks	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Red clover.....	Fertilizer	34	30	32	37	62
	Check	51	52	56	55	100
Alsike clover.....	Fertilizer	37	42	38	40	70
	Check	57	56	55	55	100
Sapling clover.....	Fertilizer	28	24	24	18	57
	Check	36	41	46	43	100
Crimson clover.....	Fertilizer	21	14	15	14	70
	Check	29	23	20	19	100
White sweet clover.....	Fertilizer	13	11	9	9	51
	Check	24	21	17	21	100
Lespedeza.	Fertilizer	40	41	48	42	65
	Check	63	71	71	57	100
White clover.....	Fertilizer	23	26	36	34	61
	Check	45	41	61	49	100
Kentucky bluegrass.....	Fertilizer	10	12	11	7	46
	Check	21	23	22	21	100
Canada bluegrass.	Fertilizer	16	15	15	20	56
	Check	29	31	30	27	100
Meadow fescue.....	Fertilizer	24	26	26	25	104
	Check	20	26	26	25	100
Sheep's fescue.....	Fertilizer	19	17	17	13	81
	Check	20	22	20	19	100
Tall meadow oat grass.....	Fertilizer	38	38	38	33	84
	Check	48	43	41	44	100
Rhode Island bent grass.....	Fertilizer	6	7	8	7	58
	Check	10	14	10	14	100
Orchard grass.....	Fertilizer	17	17	17	16	66
	Check	23	25	25	29	100

TABLE 3—*Continued*

SEEDS	TREATMENT	GERMINATION OF SEED TREATED				GERMINATION FOR ALL PERIODS OF CONTACT AS COMPARED WITH CHECK
		1 week	2 weeks	3 weeks	4 weeks	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Creeping bent grass.....	Fertilizer	20	28	31	34	59
	Check	42	47	51	52	100
Timothy.....	Fertilizer	58	70	69	67	77
	Check	87	88	85	84	100

TABLE 4
Percentage of germination of seeds sown with and without fertilizers

SEEDS	Crimson clover			Tall meadow oat grass	
	Separately	In contact	After 5 days	Separately	In contact
Method of seeding					
Germination	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>Treatment</i>					
Acid phosphate.....	54	9	24	41	38
Ammonium chloride.....	46	18	12	33	35
Ammonium nitrate.....	68	13	20	30	36
Ammonium phosphate.....	71	12	28	47	42
Ammonium sulfate.....	66	11	43	42	43
Calcined phosphate.....	61	14	50	26	31
Calcium carbonate.....	64	14	45	35	30
Calcium cyanamide.....	65	2	17	18	3
Calcium oxide.....	63	13	31	30	41
Potassium chloride.....	57	32	56	23	40
Potassium sulfate.....	49	14	58	47	31
Urea.....	65	12	14	35	22
Check.....	44	44	44	23	23

germination of the seeds, it seems that the concentration which allows seeds to germinate will not materially injure the seedlings.

SUMMARY

Under the conditions of this experiment and with the fertilizers and seeds used the following statements seem warranted:

1. The mixing of seeds and dry fertilizers together for one to four weeks does not injure the seeds to any noticeable extent.
2. The injury to germination, which occurs, takes place after the seeds are placed in moist soil and not before seeding.
3. Some fertilizers cause greater reduction in germination than others.
4. Some seeds show greater reduction in germination than others because of contact with fertilizers in the soil.

5. The injury from the fertilizers seems to be to the seed, preventing germination rather than destroying the seedling before it appears above the surface of the soil.

REFERENCES

- (1) HICKS, G. H. 1900 The germination of seeds as affected by certain chemical fertilizers. U. S. Dept. Agr. Div. Bot. Bul. 24.
- (2) SHERWIN, M. E. 1923 The effect of fertilizers on germination and seedling growth. *Jour. Amer. Soc. Agron*, 15: 66-73.
- (3) SHIVE, J. W. 1917 The effect of salt concentration on the germination of seeds. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1916: 455-457.

THE HYDROMETER AS A NEW METHOD FOR THE MECHANICAL ANALYSIS OF SOILS

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication July 6, 1926¹

The physical and, to a large extent, also the chemical characteristics of a soil, are controlled chiefly by the proportion of its particles of various sizes. Such physical and chemical properties or relationships of a soil as structure and tilth, ease of cultural operations, water-holding capacity, water-percolating capacity, capillary movement of water, evaporation of water, availability of water, wilting coefficient, heat of wetting, adsorbability and retainability of plant-food constituents, reactivity toward chemical reagents, solubility, and productivity, are all influenced largely by the size of particles present in the soil. This fundamental fact has long been recognized and has led to a tremendous amount of work on the part of various investigators in an effort to devise methods for making mechanical analyses of soils and determining the proportion of their particles of various sizes. The old standard mechanical analyses for this purpose are well known and need not be considered here. These methods, however, while giving an approximate determination of the amount of the various soil particles, are very laborious and time consuming. The desire to have a simpler, quicker and more accurate method for the determination of the physical constituents of the soil, has led many investigators, in the last few years, to undertake a study of the subject. Different methods for the purpose have been proposed. Among these recent methods may be mentioned those of Oden (5), Robinson (6), Jennings, Thomas, and Gardner (2), Johnson (3), and Keen (4).

The method of Oden is probably the most ingenious. It consists in placing the pan of a balance at the bottom of a column of soil suspensions, allowing the soil particles to settle upon this pan, and recording automatically the time required for a definite weight to settle. From an accumulation curve that is thus obtained, the size distribution of the mineral particles of soils is calculated by the application of certain mathematical formulas.

This original method of Oden has been somewhat modified by Keen, Johnson, and others.

¹ At the author's request, the priority rights of this paper were transferred to "The hydrometer as a new and rapid method for determining the colloidal content of soils," which was received for publication July 23, 1926 and which appeared in *Soil Science* 23: 319-335.

Robinson's method consists of allowing to settle in a cylindrical vessel a soil suspension of known concentration, which is then sampled with a 20-cc. pipette at different times and the dry matter determined. By this method it is claimed that the mechanical composition of a soil or clay can be derived from the determination of the concentration of the settling suspension for different values of depth time.

The method of Jennings, Thomas and Gardner, is about the same as that of Robinson with the main exception that in the former a "multiple-mouthed pipette" is employed, which would tend to make the process of sampling more accurate.

HYDROMETER METHOD

While investigations to devise methods (1) for the determination of the colloidal material in soils were being conducted, the idea occurred, that the hydrometer might be advantageously employed to estimate the colloidal, or at least the finer material in the soil, and also to measure the rate of settling of the soil particles and thus obtain a distribution curve from which the size distribution of the soil particles might be calculated.

The method was examined and from the results obtained, gives great promise for its successful application in estimating the fine material in the soil and especially in obtaining the distribution curve.

The hydrometer employed was a Zuevenne's Lactodensimeter² (plate 1) of a very large volume surface and considerable weight, both of which made it very sensitive and accurate. This particular Lactodensimeter had in its stem a thermometer, the graduations on which were used as points for comparative measurement of the density of the suspension of the different soils, or of the same soil at different periods of settling. The thermometer was graduated to 1 degree and the readings could be estimated to about 0.2 degrees. This degree of division on the scale seemed to give all the accuracy desired for practical purposes. The divisions ranged from 30 to 115 and consequently the density of wide degrees of concentration of soil suspensions could be measured.

The hydrometer was calibrated so that the density could be read directly on its divisions, by determining the reading of suspensions of various concentrations, then evaporating these suspensions down to dryness to ascertain the amount of dry material present, and then applying the necessary calculations to ascertain the amount of dry material to which each division of the hydrometer is equal per unit volume of water. The calibrations were done in very concentrated and very dilute suspensions of very colloidal clays. These extreme calibrations showed that each division on the hydrometer was equivalent to 0.849 gm. per 1000 cc. of water. Each reading on the hydrometer, therefore, could be translated directly into grams per liter of water.

The actual determinations were made by placing 75 gm. of soil in a large earthenware mortar. The soil was then washed two or three times with dis-

²Since this paper was written a new soil hydrometer has been made and a new method has been adopted for dispersing soils.

tilled water by decantation, care being taken to prevent stirring up the soil. The purpose of this washing is to eliminate the salts, and thus facilitate the deflocculation and make the soils more comparable. After the soils were washed they were gently rubbed with a pestle to break up the compound soil particles and to put the soil into the dispersed condition. The soil was then mixed with an excess of water, allowed to stand for a few seconds, and the supernatant liquid poured into a cylinder. The latter had a capacity of 1100 cc. and was $17\frac{1}{2}$ inches high and $2\frac{1}{2}$ inches in diameter. The process of rubbing, mixing with water, and decanting was continued until all the soil material in clays, or all the dispersable material, in sandy soils was dispersed. The cylinder containing the suspension was then filled with distilled water up to a certain point, shaken vigorously for about 5 minutes, and then set upon a table. The hydrometer was quickly placed in the suspension and readings were taken every minute for any length of time desired. Some times the readings were extended to several hours, but it was found that after the first hour the rate of settling is very slow.

Various quantities of soils were employed, but only the results with 75 gm. will be presented here. With this quantity of soil was used in every case 1075 cc. of water. In the calculation of the results, however, only 1000 cc. are employed, the other 75 cc. are considered to be consumed in wetting or saturating the soil.

The experiments were carried on at room temperature. The room temperature remained quite constant, and the thermometer in the hydrometer showed that the temperature of the suspension would not change much during the experiment.

Repeated trials showed that the results could be duplicated very closely or exactly, if the soil was well dispersed and if the temperature remained the same.

It was observed that the hydrometer would make small sudden jumps occasionally, as the mercury column of a thermometer does. This is probably due to a lag in the movement of the hydrometer. The irregularities thus produced, however, are so insignificant that they are hardly noticeable when the results are plotted.

It was also noticed that at the top of the hydrometer bulb or shoulder a small amount of sediment would settle. This occurred, however, only occasionally and on long standing and the amount was usually so small that it could hardly affect the results.

As is well known of course, the density of the suspension in a column of liquid varies with the depth, being greatest at the bottom of the column and smallest at the top of the column. The hydrometer reading proved to give an average of all the different densities in the column from top to bottom. Soil particles continue to influence the density and hence, the hydrometer reading, as long as they are dispersed in the liquid, but cease to exert an influence as soon as they settle out of the liquid.

The method as a whole is very simple, quick and accurate, and appears to have considerable promise. It can certainly give very good results on the rate of settling, from which the distribution curve of various sizes of particles can be worked out. It also gives promise of being able to determine, at least in an approximate way, the fine material in the soil, and possibly also the colloidal content.

TABLE 1
Rate of settling of soils as measured by the hydrometer method

Figures represent amount of dry material per liter of water staying in suspension at any given time out of 75 gm. sample taken.

TIME	OHIO SILT LOAM	TENNESSEE SILT LOAM	URBANA CLAY LOAM	CALIFORNIA YOLO CLAY LOAM	CALIFORNIA YOLO CLAY	ONTONAGON SILT LOAM A ₁	ONTONAGON SILT LOAM A ₂	ONTONAGON SILT LOAM B ₁
minutes	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0	63.053	44.375	63.053	67.289	62.204	62.204	64.751	66.449
1	52.865	41.828	58.808	60.506	57.110	57.959	58.808	63.902
2	46.073	40.300	52.865	54.563	52.865	55.412	56.261	62.204
3	42.677	38.008	49.469	50.318	50.318	53.714	54.139	61.779
4	38.432	36.055	46.073	46.922	47.347	52.865	52.865	61.355
5	36.734	34.187	43.526	45.649	46.073	51.167	51.595	61.100
6	34.187	32.574	40.979	43.526	44.800	50.318	51.167	60.506
7	32.065	30.961	39.281	41.828	54.102	49.469	50.743	60.336
8	30.791	29.518	36.734	40.555	41.828	48.620	50.063	59.826
9	29.518	28.244	35.461	39.281	40.555	47.771	49.469	59.572
10	28.074	26.546	34.187	38.008	39.451	47.347	48.875	59.402
11	26.291	25.357	32.914	36.904	38.602	46.752	48.620	58.977
12	25.273	24.678	31.640	36.564	37.753	46.243	48.196	58.638
13	23.999	23.744	30.367	35.885	36.904	45.733	47.686	58.383
14	23.150	22.726	29.518	35.036	36.310	45.394	47.092	58.128
15	22.471	22.131	28.669	34.442	35.800	45.054	46.752	57.959
16	21.877	21.452	27.820	33.763	35.036	44.630	46.243	57.789
17	21.282	20.773	26.471	32.914	34.612	44.375	46.073	57.619
18	20.003	20.433	26.122	32.489	34.187	44.205	45.818	57.534
19	19.924	19.584	25.273	31.895	33.763	43.696	45.563	57.279
20	19.584	18.990	24.848	31.470	32.914	43.441	45.394	57.194
30	16.188	14.915	20.263	27.225	28.669	40.979	44.120	56.006
60	11.159	10.160	13.896	21.367	22.216	37.753	41.828	53.459
75	10.120		12.113					
95			10.415	17.200	18.900	35.885		
240	7.014				12.113		34.612	
1260								33.763

In view of the foregoing description and discussion it would appear that the hydrometer method would have many advantages over the other methods that have been proposed. In the first place, it is simpler, much quicker, more practical and ought to be more accurate than most of the other methods, especially those of Robinson and Jennings, Thomas, and Gardner, where the suspensions have to be sampled by pipettes. The fact that the density of the

suspension column varies with the depth, that the hydrometer reading gives an average of all the densities from the top to the line where soil particles go out of suspension and settle, and also that the hydrometer reading indicates directly the amount of material that is in suspension at any particular moment, makes the method very unique indeed. Furthermore, the whole soil can be used.

TABLE 2

Rate of settling of soils as measured by the hydrometer method

Figures represent amount of dry matter per liter of water staying in suspension at any given time, out of 75 gm. sample taken.

TIME	STEWART LOAM A ₁	STEWART LOAM A ₂	STEWART LOAM B ₁	ONAWAY LOAM A ₁	ONAWAY LOAM A ₂	ONAWAY LOAM C ₁	NAPANEE SILT LOAM A ₁	NAPANEE SILT LOAM B ₁	NAPANEE SILT LOAM C ₁
minutes	gm.	gm	gm	gm.	gm	gm.	gm.	gm.	gm.
0	35 036	38 432	37 583	20.603	22 301	30 791	58 808	58 808	59.657
1	27 820	26 122	26 122	11 689	14.660	26 122	49 045	51.107	52 865
2	21 452	23 150	22.726	8 712	12.962	23.999	44 800	47.347	49 469
3	19 754	21 452	19.754	7 430	12 113	23 000	40 979	44.969	46 498
4	19 216	20 603	19 075	7.014	11 360	22 301	38 602	42.677	44.375
5	17 207	19.499	18 056		10.840	21 612	36.564	41.149	42 847
6	16.438	18 481	16 952		9 910		35 036	39 026	41 658
7	15 509	18 056	16.188		9 620		33 338	38.602	40.130
8	15 085	17.377	15.509		9 400		31 810	37 583	38.941
9	14 236	17.037	15 085		9.050		30 791	36.734	38 008
10	13 811	16.443	14 660		8 792	15 589	29 687	36 065	37.074
11	13 407	16.103	14.405		8 712		28.753	35.206	36.310
12	13 142	15 679	14 066		8 552		27.989	34.866	35 885
13	12 698	15 424	13 811		8 288		27 395	34.187	35.206
14	12 273	15 169	13 556		8.128		26 376	33 847	34.527
15	11.033	15.000	13.301		7 863		25 867	33 338	34.017
16					7.703		25.188	32.489	33 338
17					7 519		24 848	32 234	32.659
18					7.279		24.169	31.810	32.319
19					7.094	13 202	23 744	31 385	32.065
20	10.335	13 387	12.368		7.014		23 320	30 961	31.470
30	8.208	12.113	11.179				19 584	27.310	26.122
60			8.882			7 359	14.236	23 575	10.415

This work is presented as a preliminary report. Being of a preliminary nature the data have not been subjected to mathematical treatment. This will be done subsequently when the data have been checked in various ways. The distribution curve as actually obtained by the experimental data is shown, however.

EXPERIMENTAL DATA

In accordance with the hydrometer method described, the rate of settling was determined for over 30 soils ranging from sand to the heaviest type of clay.

The specific soils investigated included Hillsdale sandy loam A₁, B₁ and B₂ horizons; Chippewa fine sandy loam A₁, B₁ and B₂; Fox loam A₂, B₁ and B₂; Stewart loam A₁, A₂ and B₁; Onway loam A₁, A₂, B₁ and C₁; Napanee silt loam A₁, B₁ and C₁; Ohio silt loam; Tennessee silt loam; Michigan silt loam; Illinois clay loam; California Yolo clay loam and clay; Ontonagon silt loam A₁, A₂, and Ontonagon clay B₁. The results obtained on these various soils are presented in tables 1, 2 and 3. Because of lack of space, not all of the readings are given

TABLE 3

Rate of settling of soils as measured by the hydrometer method

Figures represent amount of dry material per liter of water, staying in suspension at any given time out of 75 gm. sample taken.

TIME	HILLSDALE SANDY LOAM A ₁	HILLSDALE SANDY LOAM B ₁	HILLSDALE SANDY LOAM B ₂	CHIPPEWA FINE SANDY LOAM A ₁	CHIPPEWA FINE SANDY LOAM B ₂	FOX LOAM A ₂	FOX LOAM B ₁	FOX LOAM B ₂
minutes	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0	25.697	20.603	21.452	49.469	49.469	28.244	30.791	17.632
1	18.481	15.509	12.113	21.877	11.26	22.726	23.999	15.085
2	16.358	14.236	10.415	15.934	4.00	19.754	21.282	13.811
3	15.085	13.387	9.137	13.387		18.056	19.584	12.962
4	13.811	12.538	7.700	11.689		16.358	18.056	11.858
5	13.132	11.689	7.439	10.400		15.509	17.037	10.585
6	12.538	11.264	7.014	9.500		14.660	15.934	8.712
7	11.773	10.840		9.100		13.811	14.830	7.014
8	11.349	10.245		8.712		12.962	13.217	
9	11.179	9.561		8.270		12.113	12.538	
10	10.585	9.137		7.014		11.264	11.264	
11	10.160	8.712				10.585	9.991	
12	9.736	8.288				10.415	8.882	
13	9.476	7.863				9.821	7.863	
14	9.137	7.863				9.137	7.014	
15	8.797	7.693				8.982		
16	8.627	7.184						
17	8.372	7.014						
18	8.203							
19	8.033							
20	7.863							
21	7.693							
22	7.439							

for some of the soils whose rate of settling was measured for many hours. To facilitate presentation, the typical data of several of the soils are graphed and shown in plate 2.

The data in tables 1, 2, and 3 together with plate 2 show unmistakably that the hydrometer can be used to measure the rate of settling of soils from which a distribution curve might be worked out. It will be seen both from the tables and from the figure that the results are regular and consistent and

that they must follow certain physical laws. The data plot into unusually smooth curves which would strongly indicate the applicability and reliability of the method.

An examination of the results more in detail shows that the rate at which the soil particles settle varies tremendously with the different soils. In some soils the rate is exceedingly rapid, in others it is very slow. In Chippewa loam, for instance, the settling is so rapid that at the end of 2 minutes there is only about 4 gm. in suspension in a liter of water out of 75 gm. of the original sample. In Ontonagon Clay B₁ at the end of 2 minutes there is 62.204 gm. in suspension out of the 75 gm., and even at the end of 21 hours there is still 33.763 gm. in suspension. As would be expected, of course, the greater the colloidal content of a soil the slower is the rate of settling and the greater the amount of material in suspension at any given time. The converse of this would also be true. There are of course some factors which sometimes tend to modify these rules.

As stated previously, no attempt will be made at this time to apply Stokes' law and other mathematical formulae to these experimental results in order to obtain the distribution curve, i.e. the sizes and the amount of the various soil particles. This will be reserved for a later date.

It is of interest to state here that there seems to be a relationship between the colloidal content of a soil as determined by the heat of wetting method and the amount of material remaining in suspension after standing a given time. This suggests the possibility that the hydrometer method might also be used to estimate the fine or colloidal material in soils.

SUMMARY

The hydrometer method is presented as a new means of measuring the rate of settling of soil particles and thus of obtaining a curve from which the distribution of the soil particles of various sizes might be calculated.

The method also gives promise of allowing an estimation of the amount of fine or colloidal material in soils.

REFERENCES

- (1) BOUYOUCOS, G. J. 1925 The heat of wetting as a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153-162.
- (2) JENNINGS, D. S., THOMAS, M. O., AND GARDNER, W. 1922 A new method of mechanical analysis of soils. *Soil Sci.* 14: 485-499.
- (3) JOHNSON, H. W. 1923 A new apparatus for mechanical analysis of soils. *Soil Sci.* 16: 363-366.
- (4) KEEN, B. A. Work not published, but referred to by Odgen (5).
- (5) ODGEN, S. 1924 Methods to determine the size distribution of soil particles. *Fourth Internat. Soil Sci. Conf.* Sect. 1-9, no. 5: 1-32.
- (6) ROBINSON, G. W. 1922 A new method for mechanical analysis of soils and other dispersions. *Jour. Agr. Sci.* 12: 306-321.

• PLATE 1

TYPE OF HYDROMETER, CYLINDER, AND MORTAR EMPLOYED

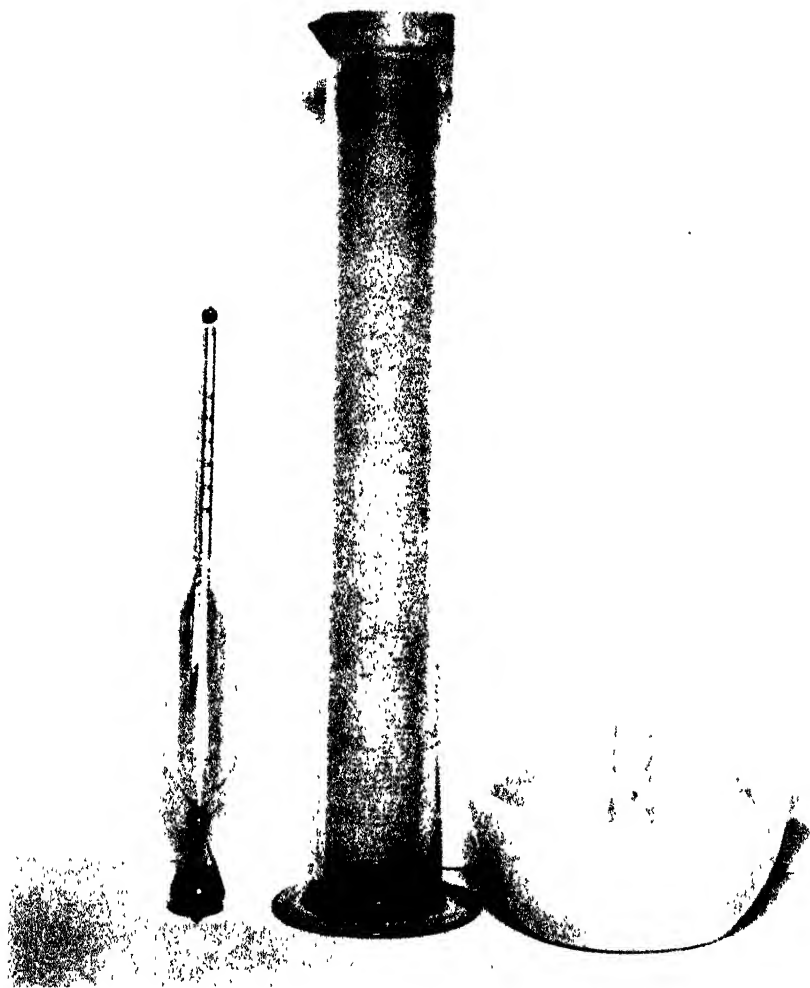
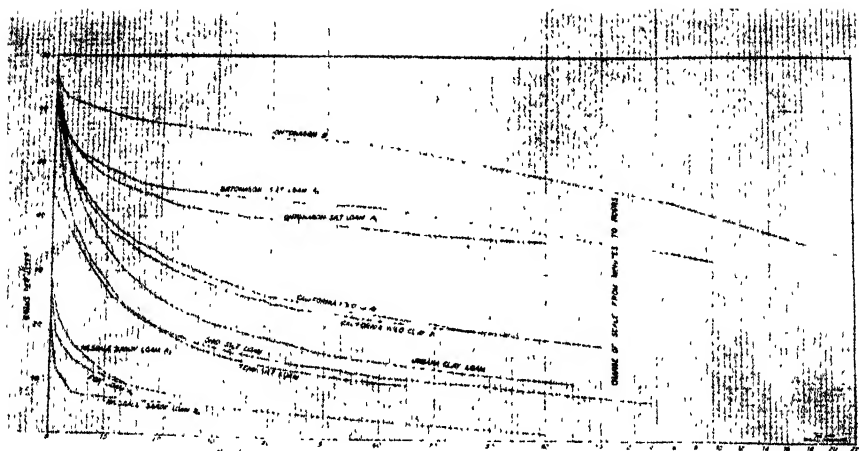




PLATE 2

CURVES SHOWING RATE OF SETTLING OF SOIL PARTICLES AS MEASURED BY HYDROMETER



EFFECT OF MOISTURE, TEMPERATURE, AND OTHER CLIMATIC CONDITIONS ON *R. LEGUMINOSARUM* IN THE SOIL¹

S. C. VANDECAVEYE

Washington Agricultural Experiment Station

Received for publication July 12, 1926

The longevity of the nodule bacteria in the soil in the absence of the host plant is still largely a matter of speculation. The earlier belief that these organisms are extremely sensitive to sunlight and desiccation is no longer acceptable, for more recent work has demonstrated that under certain conditions they are able to survive relatively wide extremes in temperature and long periods of exposure to sunlight or desiccation. The chief factors influencing the longevity of these bacteria in the soil are conceded to be the physical condition, composition, and reaction of the soil; the extremes in the temperatures of summer and winter; and the extremes in the soil moisture of the dry and wet seasons. The factors of temperature and moisture are especially significant in the semi-arid sections under cultivation.

Since in these sections crop production is largely limited by the amount of available soil nitrogen, crop rotations including legumes are rapidly being introduced for the primary purpose of maintaining the soil nitrogen supply for the production of maximum wheat yields in a permanent system of grain farming. This maintenance of soil nitrogen is so dependent on the symbiotic nitrogen fixation of the legume bacteria that it is extremely important from an economic standpoint to know that these bacteria are present and to ascertain whether they can survive successfully in the soil during the complete cycle of the crop rotation. The semi-arid section of Eastern Washington, due to its peculiar climatic conditions, with long, cold winters of freezing and thawing followed by long dry periods with relatively high temperatures during the growing season, offers an especially favorable opportunity for this study. The experiments reported in this paper are chiefly concerned with the effect of the long, dry, and hot periods of the summer and to a less extent with the cold temperatures and relatively high soil moisture during the winter on the viability of *R. leguminosarum* in the soil.

¹ Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 134, College of Agriculture and Experiment Station, State College of Washington, Pullman, Washington.

THE EFFECT OF TEMPERATURE AND EXTREMELY LOW AND HIGH SOIL MOISTURE
ON *R. LEGUMINOSARUM*

An examination of the literature pertaining to the effect of temperature and soil moisture on *R. leguminosarum* shows that most of the earlier investigators agreed that these organisms are very sensitive to sunlight and desiccation. More recent studies do not entirely support this viewpoint although results are largely variable as can be seen from the typical examples which follow:

Chester (3) found that legume bacteria perish very rapidly when dried on the seed, whereas Fellers (4) and Temple (10) observed that good nodulation was produced from inoculated seeds exposed to desiccation for 5 and 9 months. It seems reasonable to conceive that the seed coat which is generally smooth and firm offers but little protection to the bacteria and that soil particles, on account of the hygroscopic film surrounding them might give the bacteria better protection against the effects of desiccation. Even as with the seeds the results here indicate wide variations. Prucha (8) found that legume bacteria inoculated in clay, sandy loam, and sand, dried subsequently under atmospheric conditions and kept dry for 46 days, were either greatly reduced in number or in their infecting power. Fellers (4) on the other hand, was of the opinion that the symbiotic nitrogen fixing bacteria may live for years in dry soil. He found that fresh soil taken from an inoculated soybean field and stored in a dry condition for 18 months caused copious nodule production on soybean plants. Perhaps the most convincing evidence in favor of the resistance of legume bacteria to sunlight and desiccation was offered by Albrecht (1) in his studies with silt loam soil. He observed that thoroughly inoculated silt loam, taken from the field and spread in a thin layer, exposed to direct sunlight for 2 months, and later stored in a dry condition for 30 months, was as good for inoculating purposes as the fresh moist soil gathered from the field.

Apparently the temperature investigators have secured more coördinating results. Jones and Tisdale (7) reported that vigorous nodules are produced in inoculated soils at any temperature at which the plant will survive. Fred (5) stated that legume bacteria are able to survive through the winter and will withstand ordinary winter temperatures.

The foregoing examples which are typical of the many varying results in the literature on the subject are sufficient to show the need of more definite knowledge along this line.

In an attempt to throw more light on these points an experiment was undertaken to determine the effect of wide variations in temperature and moisture on the development and nodule producing power of *R. leguminosarum* in sterilized Palouse silt loam. This soil is of basaltic lava formation and is highly productive. Its nitrogen content is approximately 0.18 to 0.20 per cent, its optimum moisture capacity 20 per cent, and its reaction variation from pH 5.5 to pH 6.5. The soil in general does not readily respond to lime treatments.

Fifteen $\frac{1}{2}$ -gallon glazed pots were filled to within 1 inch of the top with soil and sterilized in the autoclave in two intermittent sterilizations of 6 hours each at 15 pounds pressure. The pots were allowed to cool and the soil moisture was adjusted to its optimum point with distilled water. The first five pots were inoculated with a suspension of a pure culture of legume bacteria for alfalfa; the second five with a similar suspension of legume bacteria for peas; and the last five with a similar suspension of legume bacteria for clover. These sus-

pensions were thoroughly mixed with the soil in the pots and allowed to stand at room temperature for 5 days. Plate counts were taken from all the pots. Then, from each series, triplicate pots were placed out of doors to approximate field conditions. The remaining two pots of each series were placed in the greenhouse under optimum moisture and temperature conditions to serve as a control. Plate counts of all the pots were made periodically to determine the effect of the various factors on the numbers of bacteria.

TABLE 1

Average counts in millions of bacteria per gram of soil from triplicate pots out of doors and duplicate pots in the greenhouse as affected by temperature and moisture

DATE OF SAMPLING	TEMPERATURE	ALFALFA ORGANISMS		PEA ORGANISMS		CLOVER ORGANISMS	
		Moisture	Number of bacteria	Moisture	Number of bacteria	Moisture	Number of bacteria
		°F.	per cent		per cent		per cent
Pots kept out of doors							
1/24	68	20.0	123.00	20.0	933.00	20.0	1,926.00
3/6	25	20.0	187.40	20.0	93.40	20.0	254.50
6/22	78	9.0	1.71	11.0	2.01	9.5	2.33
7/22	92	2.8	1.00	3.8	2.51	2.5	1.21
8/22	99	0.7	2.15	0.4	2.43	0.3	1.92
9/24	86	1.6	1.09	1.8	1.62	1.8	1.63
11/6	67	17.1	7.40	17.5	13.50	17.5	10.20
12/4	30	22.0	4.08	21.8	5.61	22.4	10.00
1/18	17	Flooded	1.59	Flooded	1.46	Flooded	1.30
4/9	14	18.0	0.442	17.8	0.408	18.1	0.353
Pots kept in greenhouse							
1/24	77	20.0	670.00	20.0	420.00	20.0	2,160.00
3/6	77	20.0	30.00	20.0	36.50	20.0	38.00
6/22	83	15.0	44.90	14.5	16.25	14.2	14.25
7/22	89	16.0	9.40	17.0	10.88	16.0	7.82
8/22	86	16.0	14.60	16.0	14.66	16.5	21.50
9/24	76	16.4	16.50	15.5	15.50	17.7	17.87
11/6	75	17.2	17.83	14.5	11.16	18.6	10.16
12/4	68	15.5	6.37	16.0	6.50	15.5	9.25
1/18	68	16.3	2.68	16.9	2.03	16.0	24.64
4/9	77	17.5	13.62	17.1	5.02	16.7	11.40

Since all the pots were exposed to contamination from the air the difficulties involved in making counts for legume bacteria alone are readily recognized, hence no claim is made that the exact numbers were determined. By using nitrogen-free agar in the plates, however, many of the contaminating forms were eliminated. In addition to this, only typical colonies of *R. leguminosarum* were counted, so the results may be said to be reliable. The samples

of soil designed for plate counts and moisture determinations were secured each time in the following manner: One-half inch of the surface soil was temporarily removed and, with a small auger, several borings were made through the remaining central portion of the soil column until sufficient soil was collected to make both determinations. The holes resulting from the borings were filled from the surrounding soil and the surface soil was replaced. Each sample was thoroughly mixed previous to weighing out 25 gm. for the moisture determination and 1 gm. for the bacterial count. The moisture was determined by allowing the samples to dry to constant weight under atmospheric conditions. The 1-gm. sample for the plate counts were shaken for 20 minutes in 99 cc. of sterile water before the usual dilutions were made. Plates were poured in quadruplicate and incubated for 7 days at 25°C. before the colonies were counted. The counts of the quadruplicate plates were averaged for each individual pot. Then the results of the triplicate pots out of doors and those of the duplicate pots in the green-house, were again averaged so that the numbers given in millions in table 1 represent the average counts of 12 plates and 8 plates respectively. The moisture and temperature determinations appear in the same table. The temperatures recorded represent the maximum temperature occurring between each two dates of sampling in the summer months, and the minimum temperature occurring between each two dates of sampling during the winter months.

The most striking feature of the experiment is that the legume bacteria inoculated in sterile soil multiplied very rapidly during the first few weeks and then decreased considerably regardless of moisture and temperature changes. This behavior may be influenced by many factors, chief of which is probably the competition of invading organisms. Under optimum moisture and temperature conditions as maintained in the greenhouse this competition seemed to establish an equilibrium wherein the legume bacteria had decreased to certain numbers, which remained fairly constant for several months. This equilibrium period was followed by a further drop in numbers which also continued for several months and was succeeded by a period during which there was a strong tendency toward the first state of equilibrium. This condition is hardly explainable on the basis of the data secured. It is conceivable that the fluctuation might be caused by a similar fluctuation in the amount of available food material, resulting from the difference in metabolic processes of the invading and the symbiotic nitrogen fixing bacteria, and the needs of the two kinds of bacteria for different food.

In the pots kept under field conditions there was a distinct correlation between the moisture content of the soil and the number of living bacteria. However, under the severest conditions of heat and drought prevailing during the months of June, July, August, and September, the reduction in numbers of viable legume bacteria was relatively small. As soon as moisture was supplied by the fall rains late in October, the bacterial numbers increased rapidly and soon reached approximately the same proportions as those in the pots in the green-

house. A rather abnormal condition occurred in the pots under field conditions when the precipitation during the winter became so great that the soil in the pots was covered with water. This was due to the fact that no drainage had been provided for in the glazed pots. This flooded condition lasted for 2 weeks and was very detrimental to the legume bacteria. In fact the reduction in their numbers due to excess moisture was much greater and lasted much longer than that caused by high summer temperatures and extremely low moisture content. The effect was still noticeable 2 months after the excess water had disappeared from the pots.

At that time—14 months after the experiment was started—sterilized seeds of the proper kinds of legumes were planted in all the pots used in the experiment, to ascertain by comparison, the effect of the apparently impaired condition of the bacterial cells of the flooded pots on their ability to produce nodules. The plants were allowed to grow for 60 days and were then examined for nodules by carefully washing the soil from the roots. Abundant nodules were found on the roots of the plants in all the pots irrespective of previous conditions. No difference could be observed between the results from the pots kept under optimum conditions in the greenhouse and those kept under the extreme hardships of field conditions. The experiment demonstrated that, in certain soils at least, legume bacteria may survive unusual exposure to wide extremes of temperature and soil moisture without any apparent effect on their ability to produce nodules on the roots of legumes.

THE LONGEVITY OF LEGUME BACTERIA IN THE SOIL IN THE ABSENCE OF THE HOST PLANT

It has been demonstrated in the previous experiment that laboratory cultures of legume bacteria are able to survive in the soil for more than a year under adverse climatic conditions. But from the economic standpoint of the farmer, it is also important to know how long they are able to survive under natural conditions in cultivated soils in the absence of the host plant. The information available in the literature on the subject is rather indefinite and variable.

Russell and Morrison (9) reported that soybean nodule bacteria are capable of living under field conditions without the host plant for a period of 17 years and that they tolerate soils with a medium to a strong acid reaction. Fred (5) stated that experience has often shown that it is unnecessary to inoculate legume seed even though no legume has been grown on the land for a number of years. Hopkins (6), on the other hand, pointed out that there is no reason to believe that any of the species of the symbiotic nitrogen gathering bacteria will live in the soil for more than a few years. He admitted that it is not definitely known just how long they live in a soil without leguminous crops, although he seemed to be quite positive that they do live for two or three years, but probably not for more than five or six years.

To obtain more definite knowledge on this point a simple experiment with soil from inoculated fields was planned. Three fields that once had been inoculated with alfalfa bacteria were located, where the alfalfa had been plowed under, 3, 8, and 13 years ago respectively. As each field was summer fallowed

during the year following the plowing of the alfalfa and nothing but cereal crops were planted subsequently, no legume plants remained to support the growth of the symbiotic nitrogen-fixing bacteria. Representative samples of soil taken to a depth of 10 inches were collected from each of these fields. Each sample was thoroughly mixed and then used to fill three 2-gallon pots. Another field was located where peas with an abundance of nodules had been grown 10 years ago and where nothing but cereal crops have been raised since that time. A composite sample similar to those taken in the alfalfa fields was collected, mixed, and used to fill three 2-gallon pots. Sterilized alfalfa and pea seeds were then planted in the proper pots and allowed to grow for 60 days. At the end of this period the soil in all of the pots was carefully washed from the roots of the plants previous to the examination for nodules. Abundant nodules were found in every case and there was no perceptible difference between the nodulation on the plants grown in soil devoid of the host plant for 2 years and that devoid of the host plant for 12 years. That these results were probably not influenced by any favorable conditions of the plant house was shown in the results with the peas. It happened that shortly after the soil sample was collected from the field, uninoculated peas were again planted in that field, and also in an adjacent field where peas had never been grown. The nodulation in the field which was inoculated 10 years ago proved to be even better than that in the pot experiment. The field where peas were grown for the first time was also examined for nodules, but only a few widely scattered plants were found to be at all inoculated. These results show that when the legume bacteria are present in the soil, they will readily form nodules on the roots of the host plant. The experiment further demonstrates that when the legume bacteria are once introduced into the Palouse silt loams of the semi-arid section of Eastern Washington they are able to live without the host plant for at least 10 or 15 years and probably for a considerably longer time. The total results of both experiments offer substantial evidence to show that the drying out of soils during long, hot, dry periods of summer is not responsible for the killing of most legume bacteria of the soil.

THE EFFECT OF DUST STORMS ON THE DISTRIBUTION OF *R. LEGUMINOSARUM*

According to Allen (2), wind storms, dust storms, and mud storms are negligible factors in the distribution of legume bacteria in Eastern Washington. These storms occur quite frequently in this section and considerable amounts of fine soil are thus moved about. It would seem that the greater the number of fields which are artificially inoculated the greater would be the possibility for inoculating new fields by means of the wind blown dust. Since the results in the two previous experiments show that the legume bacteria are able to live in the soil for many years and that they can withstand desiccation for a considerable length of time, a third experiment suggested itself to determine the extent of the distribution of these bacteria as a result of wind action. Nine 2-gallon pots were filled with Palouse silt loam and sterilized in the autoclave as

in the first experiment. When cool they were placed out of doors where they were exposed to the natural atmospheric conditions for 16 months. Then triplicate pots were planted with sterile seeds of alfalfa, peas, and clover respectively. From 5 to 10 plants were allowed to each pot and 60 days after planting the plants were examined for nodules in the usual way. The nodules were counted and the results are given in table 2.

Only two pots were free from nodules. The others had only a limited number, but this was sufficient to show that viable legume bacteria are moved about by the wind and dust storms and that inoculation is possible by means of these factors. It is doubtful whether this natural means of inoculating has been effective to any marked extent in the past, for it has been the common experience that artificial inoculation by means of inoculated soil or laboratory cultures was necessary to secure thorough inoculation in fields planted to legumes for the first time. However, a few scattered inoculated plants can generally be found in new legume fields where artificial inoculation has not been practiced. As more fields become inoculated by artificial means the effect of the

TABLE 2

Nodulation in sterilized soil subsequently exposed to wind and dust storms for 16 months

TOTAL NUMBER OF NODULES ON 5 TO 10 PLANTS			
Pot number	Alfalfa	Peas	Clover
1	0	4	3
2	9	0	5
3	4	3	2

wind and dust storms in the distribution of legume bacteria will increase proportionately. Although these factors may never be sufficiently effective to insure and maintain thorough inoculation in the soils of Eastern Washington they constitute a means of natural distribution which should not be entirely overlooked.

SUMMARY

Pot experiments carried on in the greenhouse and out of doors demonstrated that laboratory cultures of *R. leguminosarum* grown in Palouse silt loam are capable of surviving unusual exposure to wide extremes of soil moisture without any apparent effect on their ability to produce nodules on the host plants.

Excessive soil moisture to the point of saturation or flooding was much more detrimental to the life of these bacteria than extreme dryness approaching air-dry conditions.

The extremes of temperatures of winter and summer did not prove to have any injurious effect on the nodule production of the legume bacteria in the soil.

Legume bacteria for alfalfa, peas, and sweet clover, once introduced into the

semi-arid soils of Eastern Washington readily produce nodules and are able to survive in the soil for 10 to 15 years or longer in the absence of the host plant.

Legume bacteria proved to be distributed by wind and dust storms, but not to the extent of causing a thorough inoculation of legumes which are planted in the fields for the first time.

REFERENCES

- (1) ALBRECHT, W. A. 1922 Viable legume bacteria in sun-dried soil. *Jour. Amer. Soc. Agron.* 14: 49-51.
- (2) ALLEN, P. W. 1923 Factors in successful inoculation of legume crops. *Wash. Agr. Exp. Sta. Bul.* 180: 16-17.
- (3) CHESTER, F. D. 1907 The effect of desiccation on root tubercle bacteria. *Del. Agr. Exp. Sta. Bul.* 78.
- (4) FELLERS, C. R. 1919 The longevity of *B. radicola* on legume seeds. *Soil Sci.* 7: 217-223.
- (5) FRED, E. B. 1920 New farm facts. *Wis. Agr. Exp. Sta. Bul.* 323: 36.
- (6) HOPKINS, C. G. 1904 Nitrogen bacteria and legumes. *Ill. Agr. Exp. Sta. Bul.* 94.
- (7) JONES, F. R., AND TISDALE, W. B. 1921 Effect of soil temperature upon the development of nodules on the roots of certain legumes. *Jour. Agr. Res.* 22: 17-31.
- (8) PRUCHA, M. J. 1915 Physiological studies of *B. radicola* of Canada field pea. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 5: 32.
- (9) RUSSELL, H. L., AND MORRISON, F. B. 1920 Experiments in farming. *Wis. Agr. Exp. Sta. Bul.* 319: 26.
- (10) TEMPLE, J. C. 1916 Studies of *B. radicola*. *Ga. Agr. Exp. Sta. Bul.* 120.

EFFECT OF LOCAL INFLUENCES IN MODIFYING THE GENERAL ATMOSPHERIC CONDITIONS¹

ALFRED SMITH²

University of Wisconsin

Received for publication July 13, 1926

A statistical study was made of the effect of local influences in modifying the general atmospheric conditions that exist in certain sections of Wisconsin.

Central Wisconsin soils are mainly light textured, the sandy members being the most abundant. North and south of this area the soils are mainly silt loams. As there is thus a distinct sandy soil covering a large area in central Wisconsin an opportunity seemed to be provided to ascertain whether the nature of the soil there exerted any marked influence on such climatic factors as temperature and rainfall.

In 1906 Henry (5) referred to this central area as follows:

There appears to be a rather remarkable deficiency of precipitation in the vicinity of the Winnebago basin as shown by the records of Hancock, Fond du Lac, and Stevens Point, the average annual precipitation at these stations being nearly 4 inches less than that of surrounding points. It does not seem probable that such a marked difference could occur in so limited an area, especially where no marked topographical features exist, and while the accuracy of the measurements made at these stations is not questioned, the period of observations, about 10 years, is too short to determine with certainty whether this discrepancy is actual or only apparent.

The records as obtained by the United States Weather Bureau (7) at certain stations over a period of years—since their establishment up to 1920 inclusive—were studied. At Hancock, which lies in the sandy area of Central Wisconsin, the United States Weather Bureau station was located from 1903 to 1920 on a farm. Surrounding country is level and there are no bodies of water or timber near. At Madison, which lies south of Hancock in the silt loam area, from 1878 to 1920 the instruments were located on one of the buildings of the University of Wisconsin in the city of Madison. This building is on a high ridge between lakes. The third station selected was Wausau, which lies north of Hancock out of the sandy area and where the instruments were exposed on the courthouse lawn during the 22 years previous to 1921. The station at Wausau was situated a few hundred feet from the Wisconsin River surrounded by hills.

¹ Part 2 of a thesis submitted at the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The author wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professor A. R. Whitson.

² Assistant Professor Soil Technology, University of California.

A study of the data obtained during the years mentioned shows that Madison has a higher mean temperature for each month of the year than either of the other two stations, whereas Wausau has the lowest mean temperature for every month. The mean July temperature as shown by this data at Hancock was 71.7°, at Madison 72.1°, and at Wausau 68.4°.

The average rainfall during these same years was: Wausau 31.68 inches, Hancock 31.49 inches, and Madison 31.18 inches. A slight decrease in rainfall is thus indicated toward the most southern station (Madison), but the distribution was not the same at these three localities. During the early part of the growing season from April to June inclusive, the mean rainfall at Hancock was higher than at either of the other stations, but during July, August, and September it was highest at Wausau. During the months of April to September inclusive, the mean monthly rainfall was lowest at Madison. The greatest difference for the mean monthly rainfall between these stations was 0.70 inch, and that was for May when Hancock had 4.48 and Madison 3.78 inches.

Several stations were next selected in the sandy area to ascertain whether their records varied considerably from each other. The localities selected were Hancock, Wisconsin Rapids, Stevens Point, Meadow Valley, Mather, Valley Junction, and Hatfield. The length of the record for these localities varied from 17 years for Mather to 28 years at Hancock. Mather was the only station at which the mean monthly temperature deviated widely. This was no doubt due to the fact that Mather is situated in a swampy area. The mean annual temperature at Mather was 42°F. and at Hancock 44.4°F. whereas at the other stations it varied from 43.3 to 43.7°F.

Two stations at approximately equal distances from Hancock were next selected. Merrill, which lies 72.5 miles due north of Hancock, and Madison, 75 miles due south of Hancock, were chosen as being in silt loam areas, and their records compared with Hancock, which is in the sandy area. The decrease in temperature northward from Madison through Hancock to Merrill was not uniform when the mean temperatures for April to September inclusive were compared. The mean temperatures at Hancock for each month, April to September inclusive, are very near to those of Madison and not midway between those of Merrill and Madison as one might at first expect.

A like comparison was then made between the temperature records at Tomahawk, which is 95 miles north of Hancock; at Brodhead, which is 108 miles south of Hancock; and at Hancock. The mean temperatures for the growing season, April to September inclusive, at Hancock were closer to those of Brodhead than to Tomahawk, thus again indicating a higher temperature at Hancock than would be indicated by a fairly uniform decrease in temperature from the southern station to the most northern station.

In comparing temperatures for stations in Wisconsin the nature of the soil is likely to have an influence only during those months when there is no snow cover and for this reason it is felt that the comparison of the temperatures

during the growing season is the most logical. All data hereafter given, therefore, are limited to these months.

A large number of Weather Bureau stations which lie in an almost north-south line were next selected and included Brodhead, Madison, Portage, Hancock, Stevens Point, Wausau, Merrill, and Tomahawk.

In this case the temperatures in the sandy area lie close to what the normal decrease in temperature from the most southern to the most northern station would indicate them to be.

As the weather of August in Wisconsin is thought by some to be less subject to the influence of cyclones or thunderstorms than any other month it is perhaps best to discuss further the mean temperature for August in and out of the sandy area. The stations selected next were Brodhead, Madison, Portage, Hancock, Stevens Point, Wausau, Merrill, and Tomahawk running from south to north. Corrections—1°F. for every 270 feet—were made for the differences in elevation—1091 feet, at Hancock; 812 feet, at Brodhead; 1450 feet at Tomahawk. The mean August temperatures of the sandy area were decidedly above the normal mean temperature for that latitude, for the decrease in temperature from Brodhead northward to Tomahawk was not uniform, especially over the sandy area. The data used here were for the years beginning with the establishment of the stations up to 1920 inclusive (6).

A comparison of the rainfall records of stations in and out of the sandy area failed to show any decided difference. One reason for this is the shortness of the records and the occasional heavy rainfall which may come at just one of the stations. In June, 1914 at Hancock in the sandy area the total precipitation was 11.75 inches, whereas at Madison in the silt loam area south, it was 3.46, and at Merrill north of the sandy area it was 7.10 inches. It is felt by the writer that no definite conclusion can be drawn regarding the influence of the sandy nature of the soils of Central Wisconsin on the rainfall, as the length of the record is not sufficient.

As the study up to this point failed to show in the sandy area any marked difference in rainfall due to the nature of the soil, but as there may be an increase in temperature, it was decided to compare the temperatures of the stations in this sandy area with those situated in the same latitude in Wisconsin and Minnesota.

One of the earliest notices of variations from the latitude normals was taken in 1791,³ but the causes named we now consider inadequate, although the remarks are of historic interest. Hann (4) summarized the effect of land and water in modifying the normal latitudinal temperature.

The word "anomaly" first used in connection with temperature by Cotte, was taken up by Dove in his "Distribution of Heat over the Surface of the Globe." This famous work published in 1852, contained the first charts of Isanomalous Temperatures; they were dedicated to Humboldt, the inventor of isotherms. In 1878 a valuable addition was made to the subject

³ *Obs. Phys.* 39: 42, Paris 1791.

of isanomals by Teisserenc de Bort (8). In these charts the advance over Dove's work was especially noteworthy in the increased deformation of the lines of equal departure, or isanomals. The observations of the "Challenger" Expedition, made by Buchan were the basis for the publication of Batchelder's Isanomalous Temperature Charts (1). In the preparation of these charts, the records from 1870 to 1885 were used and the causes of the irregularities of temperature distribution were grouped under two heads—the irregular distribution of the land surfaces of the globe, or continental interruptions, and the circulation of the waters of the globe in fixed and natural directions, or ocean currents. The Sahara, a sandy and rocky plateau near the equator, has conditions where absorption and radiation are very high. In summer this region warms to a maximum of 16°F. above the normal of its latitude, but in winter, despite its nearness to the equator and the warm Mediterranean and Red seas, it actually falls below its latitude mean temperature, as do the similar Arabian desert nearby and the desert in Australia. In like manner Connolly (2) has shown the unequal distribution of temperature in preparing his charts on the "Annual Ranges of Temperature."

TABLE 1
Average mean temperatures 1913-1919
Corrected to elevation of 976½ feet

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F	°F.	°F.	°F.	°F.	°F	°F.
Rochester.....	45 4	55 9	65 7	71 7	68 6	59 9	61 2
Faribault.	46 3	56.7	65 7	72.0	69.0	60.5	61.7
St. Peter.....	47 9	57 6	67.1	73 2	70 3	61 3	62.9
Winnebago.....	47.0	57.7	67.5	74 0	71.0	61.5	63.1
Zumbrota.....	46 1	55 9	64.6	70.8	68 1	59.6	60.7
Meadow Valley.....	45 0	55 1	64.1	70.3	66.7	58.5	60.0
Pine River.....	45.0	55.4	64.9	70 6	67 3	59.4	60.3
Mather.....	43.1	53.7	62.8	68 9	65.0	56.7	58.4
Hancock.....	46 7	57 0	66.5	72 5	70.3	61 3	62.6
Latitude Mean.....	45.8	56 1	65.4	71.5	68.5	59.8	61.2

The records for the years 1913-1919, inclusive, were used in this study, for during this period according to the records of the United States Weather Bureau the thermometers used at the stations selected in Wisconsin and Minnesota were not moved. A longer period of years could not be used because at some of the stations the location of the thermometers was changed. All the stations selected in Wisconsin and Minnesota lie on or close to latitude 44° 8' north. The stations selected in Minnesota were Rochester, Faribault, St. Peter, Winnebago, Zumbrota; and in Wisconsin, Meadow Valley, Pine River, Mather, and Hancock. The elevation of these nine stations above sea level ranged from 840 to 1100 feet and therefore that of Meadow Valley, which was midway between the extremes at 976½ feet, was taken as the standard elevation.

As all of these stations were in approximately the same latitude the average of the records for each month is called the latitude mean and is given at the bottom of the table.

The departures of the stations located in Wisconsin from the latitude mean are shown in figure 1. The departures in the mean temperature at Hancock during the growing seasons of 1913-1919 are seen to be consistently above the latitude mean whereas those at Pine River, Meadow Valley, and Mather are below. In the immediate vicinity of the thermometers at these latter three stations there are areas of peat, muck, or swamps whereas at Hancock the soil is more predominately a sand.

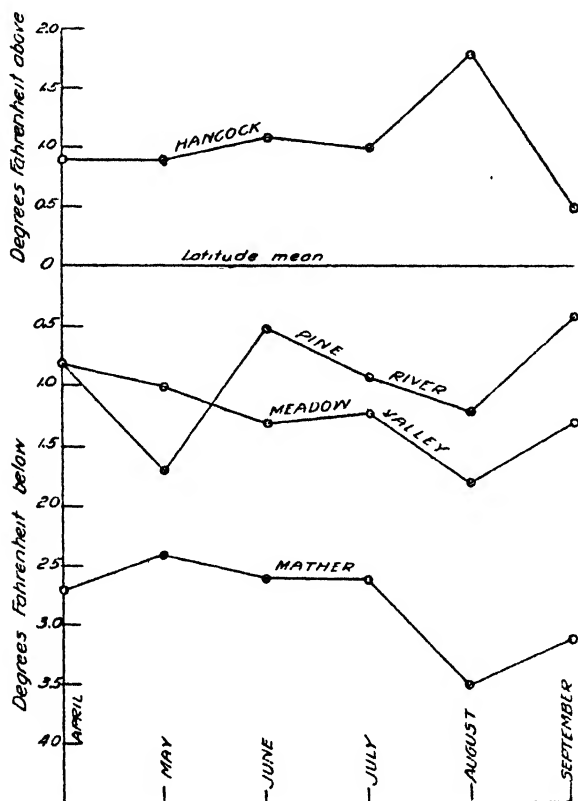


FIG. 1. MEAN TEMPERATURE RANGE DEPARTURES FOR 1913-1919

The mean maximum temperatures at these nine stations, which are approximately in the same latitude, were then compared. The data used are given in tables 2 and 3.

From the figures in the last column of table 3 it is evident that Mather has a lower mean maximum temperature during the growing season, that is from April to September inclusive, than the other stations. Hancock, on the other hand, has the highest mean maximum temperature of the Wisconsin stations and, if the mean for the growing season is taken as a basis, it is 3.3°F. higher

than at Mather. The mean maximum temperatures for the growing season for the same latitude at the Wisconsin and Minnesota stations is 88.6, whereas for Hancock it is 89.5 and for Meadow Valley 89.1°F. The departures from the latitude mean for the Wisconsin stations are shown in figure 2, which is based on table 3. A comparison of figures 1 and 2 shows that the temperatures at Hancock during the growing season were above the latitude mean whereas

TABLE 2
Mean maximum temperatures during growing seasons 1913-1919

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Rochester.....	79.0	89.3	90.7	96.3	93.0	89.9	89.7
Faribault.....	77.0	87.7	88.6	92.7	90.8	86.4	87.2
St. Peter.....	78.0	88.6	91.3	95.7	94.7	89.3	89.6
Winnebago.....	79.4	91.3	93.4	97.1	94.6	90.4	90.9
Zumbrota.....	77.0	87.3	87.0	92.4	92.1	87.0	87.3
Meadow Valley...	76.3	88.1	90.7	97.1	94.4	88.3	89.1
Pine River.....	76.3	88.0	87.9	95.9	91.9	87.3	87.9
Mather.....	74.9	84.6	86.7	93.9	91.0	86.3	86.2
Hancock.....	76.0	88.0	91.0	97.4	94.1	87.3	89.0

TABLE 3
Mean maximum temperatures during growing seasons 1913-1919
Corrected to elevation of 976½ feet

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Rochester.....	79.0	89.3	90.7	96.3	93.0	89.9	89.7
Faribault.....	77.1	87.8	88.7	92.8	90.9	86.5	87.3
St. Peter.....	77.5	88.1	90.8	95.2	94.2	88.8	89.1
Winnebago.....	79.9	91.8	93.9	97.6	95.1	90.9	91.4
Zumbrota.....	76.8	87.1	86.8	92.2	91.9	86.8	87.1
Meadow Valley.....	76.3	88.1	90.7	97.1	94.4	88.3	89.1
Pine River.....	76.0	87.7	87.6	95.6	91.6	87.0	87.6
Mather.....	74.9	84.6	86.7	93.9	91.0	86.3	86.2
Hancock.....	76.5	88.5	91.5	97.9	94.6	87.8	89.5
Latitude Mean.....	77.1	88.1	89.7	95.4	93.0	88.0	88.6

those at Mather were always below this mean. This applies to the mean temperatures for the growing season as well as to the mean maximum temperatures for the same period. In case of Pine River and Meadow Valley, these two figures are not in agreement, for in figure 1 both of these stations lie below the latitude mean whereas in figure 2 Meadow Valley is comparable to Hancock, and Pine River is more like Mather except during the month of May.

As has been stated, the Hancock Weather Bureau Station is located in the more typical sandy soils of Central Wisconsin but when this study had reached this point the writer felt that the varying results obtained at the other Wisconsin stations indicated that perhaps sufficient detailed information relative to the immediate surroundings of the thermometers was lacking. Information was obtained from the Hancock substation of the College of Agriculture,

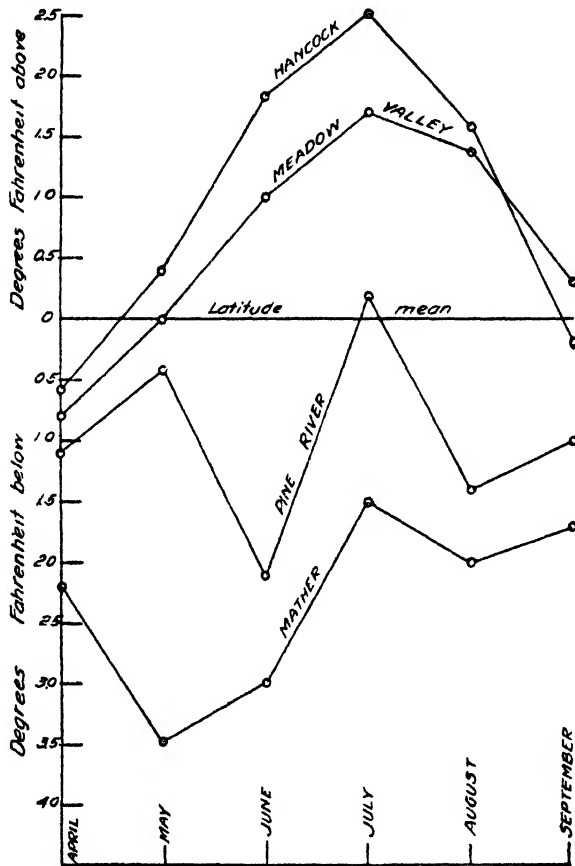


FIG. 2. MEAN MAXIMUM 1913-1919 DEPARTURE FROM LATITUDE MEAN

University of Wisconsin,⁴ which showed that the thermometers at Hancock were moved in 1910. This was not indicated in the Weather Bureau records⁵ (3).

⁴ Personal communication to the author, dated May 10, 1924.

⁵ Personal Communication to the author, dated March 20, 1924 from the Milwaukee headquarters of the Weather Bureau, United States Department of Agriculture.

In 1910 the village hall at Hancock, a brick building about 25 feet high, was built on the site occupied by the thermometers. The thermometers were then moved (1910) to the top of a lean-to about 15 feet south of the village hall and at the rear of a two-story frame building. As the previous studies were based on the information that the shelter for the thermometers was 5½ feet from the ground and later it was ascertained that this shelter was on a lean-to 15 feet above the ground it is certain that the nearness to these buildings and the distance from the ground were conditions influencing the use of temperature data so obtained.

The thermometers were moved from this lean-to in 1921 to the Hancock sub-station of the College of Agriculture, University of Wisconsin. The description of the present location (since 1921) was furnished by the United States Weather Bureau. The thermometers are now located 1088 feet above sea level; over sod 70 feet south of house; 70 feet north of barn; 50 feet east of a tree; floor of shelter is 50 inches above ground. The topography is level with a low ridge

TABLE 4
Mean temperatures for 1922, 1923

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Faribault.....	45.6	60.5	69.6	72.1	70.4	64.7	63.8
St. Peter ..	46.9	61.3	71.3	73.6	71.9	66.5	65.3
Winnebago.....	45.8	60.1	70.3	73.1	71.2	65.0	64.3
Zumbrota ..	45.2	60.0	69.2	72.0	70.5	63.8	63.5
Meadow Valley..	43.6	59.0	68.0	70.1	67.2	61.4	61.6
Pine River ..	43.7	60.1	68.3	70.1	67.9	61.5	61.9
Mather ..	42.9	58.1	67.1	69.1	66.0	60.4	60.6
Hancock ..	42.9	59.7	68.9	70.4	68.1	61.6	61.9

½ mile east; small lake 1 mile northeast; and a thin grove 5 rods west. This station is one mile due south from the station formerly in the town of Hancock and the date of the first observation was August 31, 1921.

It is unfortunate that there are only two years' records at present for the new location at Hancock; nevertheless, these might give an indication of the effect of the local conditions on temperature. The records of the eight stations which are in the same degree of latitude in Wisconsin and Minnesota were again compared by using the average of the 1922 and 1923 monthly data. Rochester, Minnesota was also previously used but could not be used again as there are no records for that station for these years. The actual mean temperature for the different months of the growing season at these nine stations is given in table 4 for the two years mentioned (3). The average for the six months is also shown in the last column. Corrections were next made, as previously, for the difference in elevation by converting to the same elevation, namely that of 976½ feet, which is the elevation of the instruments at Meadow

TABLE 5
Mean temperatures during growing seasons 1922 and 1923
 Corrected to elevation of 976½ feet

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Faribault	45.7	60.6	69.7	72.2	70.5	64.8	63.9
St. Peter	42.4	60.8	70.8	73.1	71.4	66.0	64.8
Winnebago	46.3	60.6	70.8	73.1	71.7	65.5	64.8
Zumbrota	45.0	59.8	69.0	71.8	70.3	63.6	63.3
Meadow Valley	43.6	59.0	69.0	70.1	67.2	61.4	61.6
Pine River	43.4	59.8	68.0	69.8	67.6	61.2	61.6
Mather	42.9	58.1	67.1	69.1	66.0	60.4	60.6
Hancock	43.4	60.2	69.4	70.9	68.6	62.1	62.4
Latitude Mean	44.6	59.9	69.1	71.3	69.2	63.1	62.9

TABLE 6
Mean maximum temperatures for 1922 and 1923

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F	°F	°F.	°F.	°F.	°F.	°F.
Faribault	75.5	85.0	93.5	91.5	92.5	92.0	88.3
St. Peter	77.0	85.0	96.5	96.5	95.0	97.0	91.3
Winnebago	75.0	84.5	95.5	94.0	94.5	93.0	89.4
Zumbrota	75.0	83.5	93.5	94.5	95.0	95.0	89.4
Meadow Valley	77.5	87.5	94.5	92.0	94.5	95.5	90.3
Pine River	77.5	86.0	94.0	91.0	92.0	91.5	88.7
Mather	76.5	87.0	92.0	90.5	93.5	94.0	88.9
Hancock	75.0	85.0	94.0	92.0	93.5	93.0	88.8

TABLE 7
Mean maximum temperatures for 1922 and 1923
 Corrected to elevation of 976½ feet

STATIONS	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	GROWING SEASON
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Faribault	75.6	85.1	93.6	91.6	92.6	92.1	88.4
St. Peter	76.5	84.5	96.0	96.0	94.5	96.5	90.8
Winnebago	75.5	85.0	96.0	94.5	95.0	93.5	89.9
Zumbrota	74.8	83.3	93.3	94.3	94.8	94.8	89.2
Meadow Valley	77.5	87.5	94.5	92.0	94.5	95.5	90.3
Pine River	77.2	85.7	93.7	90.7	91.7	91.2	88.4
Mather	76.5	87.0	92.0	90.5	93.5	94.0	88.9
Hancock	75.5	85.5	94.5	92.5	94.0	93.5	89.3
Latitude Mean	76.1	85.4	94.2	92.8	93.8	93.9	89.4

Valley. These corrected mean temperatures are given in table 5. The latitude mean given in table 5 is the average for the eight stations for the particular month or for the growing season. As the previous studies have shown and as this latter table likewise indicates, the temperatures at Mather were below the latitude mean for every month. The same was true during these two years at Pine River and Meadow Valley. The records in table 5, however, show that at Hancock the temperatures were generally only slightly below the latitude mean and in May and June they were slightly higher.

These eight stations were next compared by using the mean maximum temperatures for the same months during 1922 and 1923 (table 6). Corrections for differences in elevations were again made as before (table 7). There is no consistent difference between the mean maximum temperatures at Hancock and the latitude mean. During the month of August when cyclonic storms are less common in Wisconsin and Minnesota and during 1922 and 1923 the mean maximum temperature is given as 94.0 at Hancock whereas the latitude mean is slightly lower (0.20°F.).

A period of two years is too short for comparisons between stations, as slight irregularities in the instruments, inaccuracy of readings, and many other factors may serve to bring in slight discrepancies which are highly magnified in such a brief record. It was thought advisable, therefore, to study the temperature records for the month of August over a longer period of years and to ascertain if possible whether the change in the location of the instruments at Hancock had influenced the temperatures. The records from 1905 to 1923 inclusive were therefore compared at three stations in central Wisconsin—at Pine River and Meadow Valley where the thermometers were in the same locations between 1905 and 1923 inclusive, and at Hancock. In figure 3 are shown the August maximum temperatures for these three stations for the years 1905 to 1923 inclusive. The years 1920 and 1921 are omitted, as records were not obtained at all of these stations during these two years.

At Hancock from 1905 to 1910 the average August maximum temperature is shown as 91.7, from 1911 when the instruments were on the lean-to already referred to until 1919 the average August maximum temperature was 94.0 and for 1922 and 1923 when the instruments were at the Hancock substation the average was 93.5 or 0.5°F. less than when they were on the lean-to. In comparison with Hancock, the average August maximum temperature at Pine River from 1905 to 1910 was 90.7; from 1911 to 1919 it was 91.4; and for 1922 and 1923 it was 92.0°F. These latter figures show a progressive increase during the three periods chosen. The average August maximum temperature at Meadow Valley during the first period, 1905–1910, was 93.7, from 1911 to 1919 it was 93.4, and during the last period, 1922 and 1923, it was 94.5°F.

A comparison of the third period with the second shows that at Pine River as well as Meadow Valley the average August maximum temperature was higher than during the first or second periods. At Hancock the average August maximum temperature during the third period was 0.5°F. lower than

during the second period but the average of the second period was 2.3°F . higher than during the first period.

If the average August maximum temperatures for the third period are compared with the first period it is found that at all three stations it was highest during the third period to the following extent, Hancock 1.8, Pine River 1.3, and Meadow Valley 0.8°F . That during the years 1911 to 1919 inclusive, the thermometers at Hancock were on a lean-to seems to be reflected in the records

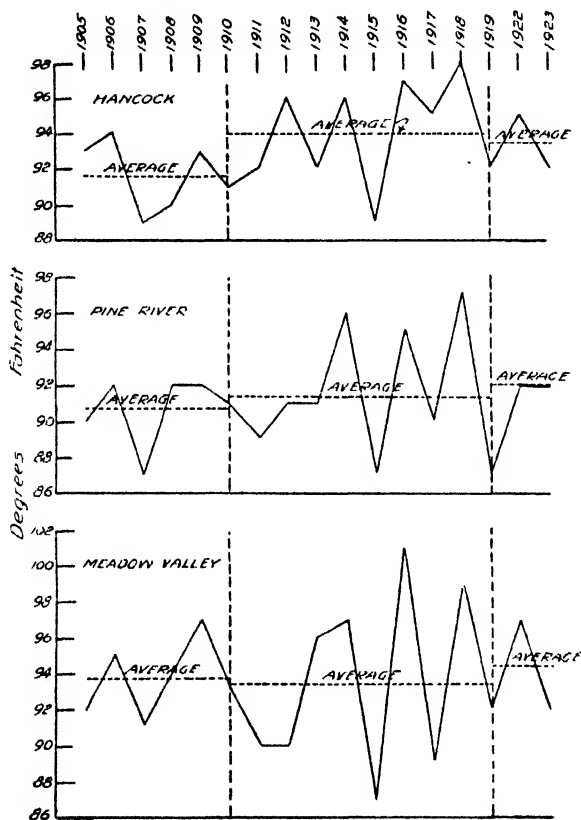


FIG 3. AUGUST MAXIMUM TEMPERATURES

obtained, for the average for these years at Hancock is much higher than during the previous period 1905 to 1910 when they were further away from the building and over sod. The radiation and reflection of heat from the lean-to and main building would result in a higher maximum temperature being recorded. During the years 1911 to 1919 inclusive, the records obtained at the other two stations do not show such a large increase in temperature over the first period, in fact one, namely Meadow Valley, shows a decrease.

That the average of the third period (1922, 1923) at Hancock is only slightly lower than the average for the second period whereas at the other two stations the average August maximum temperature for the third period is higher than for the second period is further evidence that the average for the second period at Hancock is higher than if the instruments were placed away from buildings and close to the ground.

The average for the third period at Hancock when the instruments were better located, from the writer's viewpoint, is 1.8°F . higher than for the first

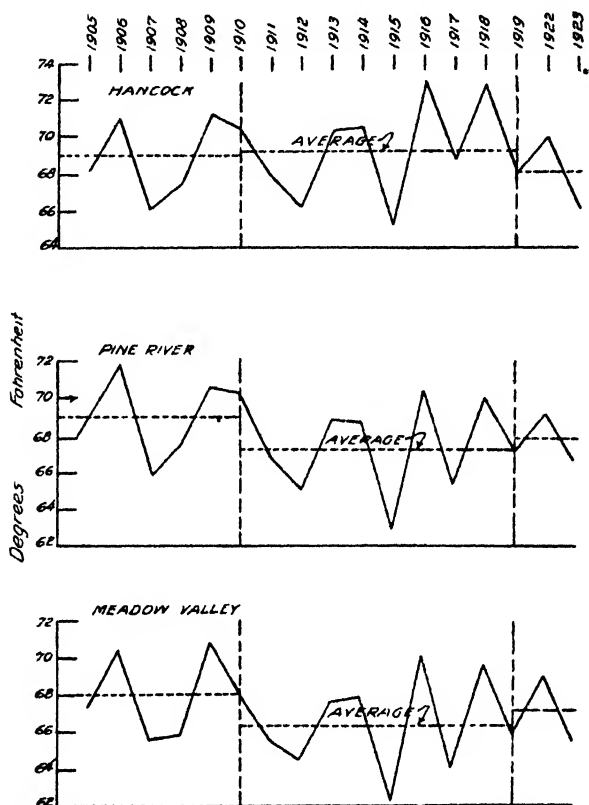


FIG. 4. MEAN AUGUST TEMPERATURES

period. This is a greater difference than was the case at the other two stations, which seems to indicate that the sandy nature of the soil at Hancock has the effect of producing a slightly higher air temperature. As previously stated, at the other two stations there are areas of peat and muck as well as sand, which, on account of their high moisture content, would offset any affect the drier sand might have in increasing the air temperatures.

A further comparison of the temperature records for these three stations was made by using the mean August temperature for the same years as for the

preceding study. The results are shown graphically in figure 4. The curves for the three stations are remarkably uniform. At Pine River the average of the mean August temperatures for the second period 1910-1919 was 67.2°F., or 1.8° lower than for the first period 1905-1910. The same was true at Meadow Valley where the average for the second period was 66.3 or 1.7°F. lower than for the first period. If the same comparison is made for Hancock the results are different, for the average of the second period there was 69.2 which was 0.2°F. higher than the average of the first period. This seems to indicate that the average for the second period was higher than it should have been.

If the average for the third period 1922, 1923 is compared with that for the first period 1905-1910 it will be seen that at each of the three stations the average for the first period was the highest. At Hancock, where the average for the first period was 69, at Pine River 69, and at Meadow Valley 68, the average for the third period at each of these stations was 68.1, 67.8, and 67.2°F. respectively.

In like manner if the third period is compared with the second period it is evident that at Hancock the average of the third period is below that for the second period whereas at the other two stations the average of the third period is above that for the second period.

The results given seem to the writer to be sufficient to show that during the period 1910 to 1919 inclusive when the thermometers at Hancock were on the lean-to previously referred to, the records obtained were higher than would have been the case if the shelter for the instruments had been placed further away from any building. In the future after a longer record has been obtained at Hancock with the instruments in their present location on the substation Farm of the College of Agriculture, a more accurate comparison will be possible between this station and others favorably situated in the sandy area as well as in the silt loam areas north and south of the sandy area.

SUMMARY

A comparison of the rainfall and temperature data obtained by the United States Weather Bureau at stations located in Wisconsin and Minnesota made over a varying period of years, for the same period, for the growing season and for individual months to ascertain whether the sandy nature of the soil in Central Wisconsin would influence these climatic elements, showed that the effect was very small.

If the conditions of sandiness and relative lack of cover do affect temperature at all, the effect is too small to be shown in the observations made by the Weather Bureau. If it were possible to place thermometers with greater care and in better locations for the purpose, it might be possible to show a small effect of soil texture on climate.

The placing of a thermometer in a shelter house on a sheet iron lean-to resulted in raising the temperature reported for the vicinity of Hancock, which

is in the sandy area of Central Wisconsin. At those stations in whose vicinity there were large bodies of water, the effect of the water in reducing the average temperatures during the warmer period of the year is clearly shown.

Any effect that the sandy nature of the soil in Central Wisconsin might have on the rainfall of this section can not be detected in the data that are at present available. Although this sandy soil does have a low water holding capacity, it has as good an opportunity of obtaining water as the heavier soils have, and the sandy nature of the soil does not tend to raise the air temperature unduly.

REFERENCES

- (1) BATCHELDER, S. F. 1894 A new series of Isanomalous Temperature Charts Based on Buchan's Isothermal Charts. *Amer. Met. Jour.* 10: 451-474.
- (2) CONNOLLY, J. L. S. 1894 A new Chart of Equal Ranges of Temperatures. *Amer. Met. Jour.* 10: 505-506.
- (3) CONNOLLY, J. L. S. 1913-1919 Annual Summaries, Minnesota Section of the Climatological Service. *U. S. Dept. Agr. Weather Bur.*
- (4) HANN, JULIUS 1883 Handbuch der Klimatologie, p. 79.
- (5) HENRY, A. J. 1906 Climatology of the United States. *U. S. Dept. Agr. Weather Bur. Bul.* Q: 529.
- (6) STEWART, W. P. 1913-1920 Annual Summaries, Wisconsin Section of the Climatological Service. *U. S. Dept. Agr. Weather Bur.*
- (7) STEWART, W. P. 1922 Summary of the Climatological Data for the United States, Sections 58, 59, 60. *U. S. Dept. Agr. Weather Bur.*
- (8) TEISSERENC DE BORT, LÉON 1879 Annales du Bureau Central Météorologique, pt. 4. Paris.

METHOD OF PREPARATION OF SOIL SUSPENSIONS AND DEGREE OF DISPERSION AS MEASURED BY THE WIEGNER-GESSNER APPARATUS¹

GEORG WIEGNER

Eidgenössische Technische Hochschule, Zurich, Switzerland²

Received for publication May 27, 1926

INTRODUCTION AND STATEMENT OF PROBLEM

The purpose of the mechanical soil analysis is to obtain the degree of dispersion of a soil in water suspension. The method of preparation and the apparatus to be employed for such analyses depend upon the purpose for which the analyses are made. If the relationship between the colloidal fraction of the soil and its content in exchangeable cations is sought, then the basis of the mechanical analyses must rest upon the complete dispersion of all colloidal material within certain set dimensions. Perhaps this may be reached only by such methods as are employed by Hissink in his use of the Atterberg and Kopecky methods. Again, if one desires to study the dispersion of a soil under more natural conditions, then a method such as the continuous registering device of Odén, or the apparatus of Wiegner, is preferable. It is not impossible that the manipulation of such refined methods—and the preparation of the soil sample for analysis—may reach the ultimate dispersion of all material in the colloidal state in the soil. Hence, despite numerous and fundamental investigations, a yet unsolved problem, in the mind of the author, is the question of the method of preparing the soil sample for the mechanical analysis.

The degree of dispersion of a soil is largely dependent on the presence of very small amounts of electrolytes which are contained in the soil and in the water. In the sedimentation apparatus of Wiegner-Gessner (3, 7), it is easy to show that as little as $\frac{1}{10,000}$ to $\frac{1}{100,000}$ normal electrolyte solutions influence the dispersion of certain soil particles. The sensitiveness differs according to the size of the particle and according to its chemical nature. From investigations carried out by R. Gallay (2), in special cases, the fraction between 5 to 25 μ in diameter is especially sensitive. The clay particles change their

¹ Translated and arranged by R. M. Barnette, Assistant Chemist, Soils, University of Florida, Agricultural Experiment Station, Gainesville.

² Agricultural Laboratory. From experiments made in coöperation with R. Gallay, Aeg. Tschudi, and R. M. Barnette.

flocculation value toward a definite electrolyte according to the nature of the adsorbed cation. Lithium-, sodium-, ammonium-, potassium-, rubidium- and caesium-clays are in the order named increasingly sensitive toward coagulation; correspondingly, the electrolyte sensitiveness increases in the series magnesium-, calcium-, strontium-, and barium-clays—in clays with two-valenced cations. The sensitiveness concurs with the so-called ion-hydration, which is correlated with the atom structure (8). The electrolyte content of the dispersing medium, together with the nature of the cations of the outer ion-sheath of clay, determines the magnitude of the electrical potential of the double-sheath of the suspended particle. Two cases are now to be differentiated:

1. If the potential of a part of the particles is under a critical value of definite magnitude at a certain electrolyte content, then a part of these clay particles adheres by collision, caused either by the Brownian movement of the smaller, or sedimentation movement of the larger, particles; and a partial or slow coagulation starts. If all the particles have a potential under the critical value, then every collision of particles becomes active, resulting in a so-called rapid or complete coagulation.
2. If the individual particles are charged above the critical potential, as may be the case in soil suspensions low in electrolyte content, then the collisions of the particles, caused either by the Brownian movement or by sedimentation, are so elastic from electrical repulsion, that no mutual adhesion and no coagulation result. The energy of electrostatic repulsion overcomes that of the mechanical collision. The suspension does not flocculate and the individual particles remain as such.

The methods of preparation, as used to date, change, on the one hand, the number of collisions of particles through increased movement (physical preparation by shaking, cooking, rubbing); or on the other hand, they change the potential (addition of a chemical, as ammonia).

The first group of physical influences must work differently depending on whether the particles in the dispersing medium, water, are charged above or below the critical potential. If extremely small amounts of foreign electrolytes are present, then the particles are charged above the critical potential, and the addition of kinetic energy, i.e., through cooking, causes dispersion. If, on the other hand, there are enough electrolytes in the dispersing medium that the particles have a smaller potential than the critical; then the increase of particle collisions through cooking, etc. decreases the dispersion of the suspension. Therefore the same method of mechanical preparation may lead to opposite final effects depending upon the electrolyte content and the magnitude of the charge on the particles. For example, cooking may disperse soil suspensions low in electrolyte content with particles above the critical potential, or it may coagulate suspensions with high electrolyte content and with particles below the critical potential. As a result, there is no absolutely effective way of preparation and every mechanical preparation is in effect dependent upon the electrolytes present in the suspension.

EXPERIMENTAL RESULTS

THE DEGREE OF DISPERSION OF A SOIL SUSPENSION PREPARED BY SHAKING, BY RUBBING, OR BY COOKING³

The following data of R. Gallay (2) demonstrate that the electrolytes in the soil suspension have an influence on the effect of shaking, rubbing, or cooking as preparation methods. The same sample of a clay soil was subjected to the following treatments: 1. Six hours shaking in machine; 2. One hour rubbing by the Beam (1) method; 3. Cooking one hour with reflux condenser.

The same soil sample is always used, an advantage which this apparatus alone offers; in that the individual soil fractions are not isolated, but merely registered. One may repeat each experiment on the same sample as often as desired. The total range of dispersion was taken by the registering device of Gessner (3).⁴ The tables are calculations from the sedimentation curves. The soil was first investigated without washing. Sixty grams of soil was shaken with distilled water by method 1, afterward the sample was cooked for one hour with a reflux condenser by method 3. The data of table 1 were calculated from the dispersion curves. The cooking here resulted in the flocculation of the finest fraction of the soil. The particles of the finer portion of the soil, which had not been freed from the electrolytes, was probably charged under the critical potential. The cooking increased the activity of the Brownian movement which increased the number of collisions, this in turn led to a partial mutual agglomeration. The number of smallest particles was decreased thereby. Therefore cooking coagulated the suspension.

The action of cooking is different if the soil is freed from electrolytes. Then the particles have a potential that is greater than the critical. The movement of particles, increased by cooking, leads to a mechanical dispersion; the potential is further increased by the hydrolysis of the clay. The experiment with the sample of the soil which had been washed free from electrolytes confirms this conjecture.

A sample of the same soil, which was coagulated formerly by cooking, was washed with distilled water for several days through an unglazed porcelain filter. The washing was carried out here, and in all other cases, in such a manner that the filter cup was placed in the soil suspension; and the dispersing medium drawn from outside into the cup and away by suction. This washed soil was shaken in the machine for 6 hours as in method 1; then the same sample, from which the water was evaporated, was rubbed, as in method 2; finally the

³ Experiments by R. Gallay.

⁴ A comprehensive description of the device together with typical sedimentation curves and the methods of calculation are given by Gessner (4). Odén (6) also gives a review of this method and also discusses Wiegner's method of calculation. The sedimentation curves obtained in these experiments are filed at the Agricultural Chemical Laboratory of the Eidgenössische Technische Hochschule. Because of the large space requirements, reproduction of the curves and their solution is not practicable.

TABLE 1

*The degree of dispersion of an unwashed clay soil, prepared by shaking and cooking**

DIAMETER OF PARTICLES	METHOD 1. SHAKING FOR 6 HOURS	METHOD 3. COOKING FOR 1 HOUR WITH REFLEX CONDENSER
mm.	per cent	per cent
Under 0.02	47.0	42.1
0.05-0.02	34.1	37.8
0.1 -0.05	13.3	13.0
0.2 -0.1	2.9	2.5
2.0 -0.2	1.8	2.1
Total.....	99.1	97.5

* Data calculated from the registered sedimentation curves.

TABLE 2

*The degree of dispersion of the clay soil used for the data in table 1, washed, then the same sample shaken, then rubbed, and finally cooked**

DIAMETER OF PARTICLES	METHOD 1 SHAKEN	METHODS 1 AND 2 SHAKEN, THEN RUBBED	METHODS 1, 2, AND 3 SHAKEN, RUBBED, COOKED
mm.	per cent	per cent	per cent
Under 0.02	49.0	53.7	54.9
0.05-0.02	33.6	31.1	29.3
0.1 -0.05	11.5	8.9	8.8
0.2 -0.1	3.0	3.3	3.5
2.0 -0.2	2.4	2.8	3.0
Total.....	99.5	99.8	99.5

* Data calculated from the registered sedimentation curves.

TABLE 3

*The degree of dispersion of a clay soil used for the data in table 2, washed, then the sample cooked then rubbed, and finally shaken**

DIAMETER OF PARTICLES	METHOD 3. COOKED	METHODS 3 AND 2. COOKED, THEN RUBBED	METHODS 3, 2, AND 1. COOKED, RUBBED, SHAKEN
mm.	per cent	per cent	per cent
Under 0.02	54.1	53.8	54.3
0.05-0.02	30.4	30.4	30.8
0.1 -0.05	8.7	9.0	8.5
0.2 -0.1	2.8	3.1	3.1
2.0 -0.2	3.5	3.4	3.7
Total.....	99.5	99.7	100.4

* Data calculated from the registered sedimentation curves.

sample was cooked for 1 hour with a reflux condenser, as in method 3. For each treatment, the settling curves were made photographically. The data of table 2 are calculated from the dispersion curves.

The comparison of the finest fraction under 0.02 mm. shows that the same sample, which was shaken, was further dispersed by rubbing and that it acquired the highest dispersion by cooking.

Another sample of the same washed clay soil was so treated that it was cooked for 1 hour with a reflux condenser (method 3); then it was rubbed (method 2); and finally it was shaken for 6 hours (method 1). Thus the order of treatment was reversed. For every treatment the settling curve was made photographically. If the cooking was most effective, it must display the strongest action in the beginning; the subsequent treatments of rubbing and shaking should not increase the degree of dispersion. This is the case, as is shown in table 3.

TABLE 4

*The degree of dispersion of the same clay soil as used for data in tables 2 and 3. Washed, and the same sample rubbed, then shaken, and finally cooked**

DIAMETER OF PARTICLES	METHOD 2. RUBBED	METHODS 2 AND 1 RUBBED, THEN SHAKEN	METHODS 2, 1 AND 3. RUBBED, SHAKEN, COOKED
mm.	per cent	per cent	per cent
Under 0.02	53.1	53.0	56.1
0.05-0.02	31.2	32.0	29.3
0.1-0.05	9.3	9.5	9.2
0.2-0.1	3.5	3.1	2.9
2.0-0.2	2.2	2.4	2.7
Total.....	99.0	100.0	100.2

* Data calculated from the registered sedimentation curves.

The cooking gave such an energetic dispersion of the washed soil, whose particles were completely charged above the critical potential, that the other treatments could exercise no further dispersing action.

Finally a third sample of the washed soil was rubbed (method 2), then the sample was shaken for 6 hours (method 1), and finally it was cooked for 1 hour with a reflux condenser (method 3). The maximum dispersion should occur with the last treatment, which was the case, as may be observed from table 4.

From these experiments, shaking as a method of preparation was more effective than cooking, for unwashed soil with higher electrolyte content and with potentials below the critical. In washed soils with low electrolyte content where the particle potential was greater than the critical, the dispersing action of cooking was more effective than that of rubbing and shaking. The action of a method of preparation is then largely dependent upon the electrolyte content of the soil and of the solution. The final effect may be contrary

for a single method, depending upon whether the soil particles are charged above or below the critical potential.

THE DEGREE OF DISPERSION OF A SOIL SUSPENSION PREPARED BY FREEZING⁵

Another physical influence which changes the degree of dispersion of a suspension is freezing. Although we scarcely apply it as a method of preparation for the mechanical soil analysis, freezing has, however, a practical significance for the estimation of the action of frost on soils. Further, the action of freezing is of theoretical interest. From the above it is to be expected that freezing would act for the most part in the same manner as an increase in the content of electrolytes; that is to say, coagulating, presupposing that enough electrolytes are present. With freezing, pure water separates, and the mother liquid is enriched in salts. The freezing should then cause flocculation in soils containing electrolytes. If, on the other hand, the electrolytes are carefully removed by washing, then the freezing should be without action, as no cause is given for the coagulation and as the particles charge themselves above the critical potential in the dispersing medium with a low content of electrolytes.

Experiments confirmed these predictions. The plan of experiment was as follows:

A soil sample of 100 gm., which had not been washed, was cooked with 600 cc. of water for $\frac{1}{2}$ hour for the purpose of dispersing it; and then investigated in the Wiegner apparatus with the registering device of Gessner (method 1). After this, the same sample was frozen and the degree of dispersion measured again (method 2). The same sample was then washed with distilled water through an unglazed porcelain filter for 8 days, cooked for $\frac{1}{2}$ hour and the degree of dispersion taken (method 3). Finally it was frozen again (method 4).

To the washed soil was added 0.001 *N* calcium chloride, and the curve for the dispersion taken (method 5). This sample containing the salt was finally frozen, and the degree of dispersion again registered (method 6).

A comparison of the data obtained with methods 1 and 2 should give the action of freezing on a soil which had not been washed; a comparison of data from methods 3 and 4, the action of freezing on the same but washed soil low in electrolyte content; a comparison of data from methods 5 and 6, the action of freezing on the same soil at a definite salt content (0.001 *N* CaCl_2). The data of table 5 show that the freezing had a coagulating action on the finer particles of the unwashed soil.

From table 6 the two dispersion curves of the same sample appear the same before and after the freezing of the washed soil, as was to be expected. The freezing had no influence on the degree of dispersion of the washed soil containing no electrolytes.

The effect of the addition of CaCl_2 is given in table 7. The soil has become coarser in degree of dispersion through the addition of CaCl_2 (com-

⁵ Experiments by Aegidius Tschudi.

TABLE 5

*The influence of freezing on the dispersion of an unwashed Loess loam from Gönhard by Aarau**

One hundred grams cooked with 600 cc. of water. The degree of dispersion determined directly and after freezing.

DIAMETER OF PARTICLES	METHOD 1. DIRECT	METHOD 2. AFTER FREEZING
mm.	per cent	per cent
Under 0.02	40.0	27.5
0.05-0.02	40.0	50.0
0.1 -0.05	13.0	15.0
0.2 -0.1	5.0	4.5
2.0 -0.2	1.3	2.0
Total.....	99.3	99.0

* Data calculated from the registered sedimentation curves.

TABLE 6

*The influence of freezing on the degree of dispersion of a washed soil**

The same sample of Loess loam soil as used for the data of table 5. Washed 8 days with distilled water; the degree of dispersion determined directly and after freezing.

DIAMETER OF PARTICLES	METHOD 3. AFTER WASHING DIRECTLY	METHOD 4. AFTER WASHING AND FREEZING
mm.	per cent	per cent
Under 0.02	50.0	50.0
0.05-0.02	30.4	30.4
0.1 -0.05	13.0	13.0
0.2 -0.1	5.0	5.0
2.0 -0.2	1.3	1.3
Total.....	99.7	99.7

* Data calculated from the registered sedimentation curves.

TABLE 7

*The influence of freezing on the degree of dispersion of a salt-containing soil**

A Loess loam, washed for 8 days, then cooked, was treated with 0.001 N CaCl_2 and the degree of dispersion measured before and after freezing.

DIAMETER OF PARTICLES	METHOD 5. WASHED SOIL IN 0.001 N CaCl_2	METHOD 6. WASHED SOIL IN 0.001 N CaCl_2 AFTER FREEZING
mm.	per cent	per cent
Under 0.02	30.5	0.0
0.05-0.02	57.3	71.0
0.1 -0.05	10.8	22.0
0.2 -0.1	1.9	6.1
2.0 -0.2	0.0	1.2
Total.....	100.5	100.3

* Data calculated from the registered sedimentation curves.

paring left hand columns in tables 6 and 7, or method 3 with method 5). The finer particles decreased about 20 per cent through the CaCl_2 addition. The freezing in the presence of small amounts of calcium salts was so strongly flocculating that particles under 0.02 mm. diameter were no longer present in the frozen lime-containing soil suspension. Also cooking the frozen soil for $\frac{1}{2}$ hour did not bring back the original degree of dispersion, as special experiments showed. In practical agriculture, we attribute to the frost a favorable flocculating action on the soil. The investigations on the suspensions show that the presence of electrolytes is one of the preliminary conditions for favorable end effects.

On the basis of these investigations it follows that the mechanical and physical methods of preparation may act entirely differently in a soil suspension, depending upon the electrolyte content of the soil. This content is the result of the degree of soil washing. It was demonstrated for example; that, the cooking was dispersing on washed soil low in electrolyte content; conversely, it was coagulating upon unwashed soil high in electrolyte content. This action is dependent upon the critical potential of the particles. As another example, freezing was coagulating on unwashed suspensions high in electrolyte content; whereas, on the other hand, it had no detectable action on dispersions low in electrolytes.

It is extraordinarily difficult to determine a definite end point for the washing with distilled water. The longer the soil is washed, the further the finest clay portions of the suspensions are hydrolyzed. Hydrogen ions replace alkali and alkaline earth ions in the clay, upon which depend changes in the swelling and imbibition, which in turn influence the size of the particles. There is then a point where the critical potential of the clay particles will be exceeded. We should exceed it in case we do not desire to measure coarse secondary aggregates; but on the other side of the critical point, the washing is increasingly active in changing the degree of dispersion.

THE DEGREE OF DISPERSION OF CARBONATE-FREE AND HUMUS-FREE SOIL SUSPENSIONS (SOIL 1458) AND OF CARBONATE-CONTAINING AND HUMUS-CONTAINING SOIL SUSPENSIONS (SOIL 1459)⁶

The experimental plan

In order to investigate the action of chemical additions, as of ammonia, which cause the increase of potential and increase of dispersion, measurements were carried out on the degree of dispersion in carbonate-free and humus-free soil sample 1458, and carbonate-containing and humus-containing sample 1459.⁶ Placed at our disposal by *Dr. Hissink* of Groningen. The carbonate-containing sample had, according to the report of Novak (5) from 7.6 to 9.3 per cent calcium carbonate. The following plan of investigation

⁶ Experiments by R. M. Barnette.

was used: the carbonate-free and humus-free soil sample *A* 1458 was investigated—the same sample being used—first after being cooked with water, then after it was thoroughly washed, and finally treated with increasing amounts of ammonia for the purpose of dispersing the particles.

The carbonate-containing and humus-containing soil sample (1459) was investigated, first, after being cooked with water, then after it was thoroughly washed, in order to investigate the effect of the dissolution of CaCO_3 by water.

Another sample *B* of the lime-containing sample (1459) was investigated, first, after being cooked, and then with increasing amounts of ammonia for dispersing the particles.

Finally a third sample *C* of the lime-containing soil (1459) was treated in the cold with a quantity of dilute HCl equivalent to the amount of CaCO_3 , then thoroughly washed and finally dispersed with increasing amounts of ammonia. Every operation was controlled by registering the dispersion curve photographically.

The preparation of the samples was as follows:

1. Sample A of the carbonate-free Dutch soil (1458).

a. Soil sample *A* (100 gm. in 200 cc. of water) was cooked for $\frac{1}{4}$ hour; after cooling, it was diluted with water, and the degree of dispersion measured directly in the Wiegner apparatus with Gessner's registering device (method 1).

b. The same sample *A* was washed for three weeks with distilled water through an unglazed porcelain filter (method 2). The test for lime was still positive.

2. Sample B of the carbonate-containing Dutch soil (1459).

a. Sample *B* of the carbonate-containing soil (100 gm. in 200 cc.) was cooked for $\frac{1}{4}$ hour; after being cooled, the suspension was diluted with water and investigated directly (method 1).

b. The same sample *B*, unwashed, was treated with ammonia until the final content was equivalent to 0.01 (method 5).

c. The same sample *B*, unwashed, was raised to an ammonia content of 0.1 normal (method 6).

d. The same soil sample *B*, unwashed, was raised to an ammonia content of 0.5 normal (method 7).

3. Sample C of the carbonate-containing Dutch soil (1459).

a. A 100-gm. sample *C* of the carbonate-containing Dutch soil was treated with dilute hydrochloric acid approximately equivalent to the amount of calcium carbonate present. The sample was then washed for 3 weeks through an unglazed porcelain filter. The test for lime was still positive.

b. The sample *C* was treated with ammonia to a 0.1 normal content (method 9).

c. The same sample *C* was raised to an ammonia content of 0.5 normal (method 10).

The degree of dispersion of a carbonate-free soil suspension prepared by washing and by the addition of ammonia to the washed soil

1. Sample A of the carbonate-free Dutch soil (1458).

a. Table 8 gives the data calculated from the sedimentation curves of the cooked, the washed, and the ammonia-treated soil.

The figures of the second and third columns show that the removal of electrolytes from the soil was strongly dispersing. The addition of ammonia was

coagulating on the finer part of the washed soil (the percentage of the particles under 4μ obtained by methods 3 and 4 is smaller than from method 2); while ammonia was dispersing, if we choose the dispersion curve of the unwashed soil as a basis of comparison (the fraction under 4μ for methods 3 and 4, is greater than for method 1). We have here again the result that the influence of a method of preparation (in this case, the addition of ammonia) is different depending upon the electrolyte content of the sample. Ammonia was dispersing as long as the particles were under the critical potential.

TABLE 8

The influence of washing and the addition of ammonia on the degree of dispersion of a cooked carbonate-free Dutch soil (Sample A 1458)*

DIAMETER OF PARTICLES	METHOD 1. SAMPLE A COOKED	METHOD 2. SAME SAMPLE WASHED FOR 3 WEEKS	METHOD 3. SAME WASHED SAMPLE IN 0.1 N AMMONIA	METHOD 4. SAME WASHED SAMPLE IN 0.5 N AMMONIA
mm.	per cent	per cent	per cent	per cent
Under 0.004	42.89	69.70	61.42	59.46
0.006-0.004	17.40	5.15	14.28	15.75
0.008-0.006	7.53	2.73	2.28	2.53
0.010-0.008	3.24	3.03	2.00	2.85
0.012-0.010	2.85	1.82	5.14	2.57
0.014-0.012	4.67	3.03		2.85
0.016-0.014	2.86		4.28	1.43
0.018-0.016	2.59	1.21	2.57	2.57
0.020-0.018		0.60		2.28
0.030-0.020	5.97	9.04	2.86	3.14
0.040-0.030	6.25	1.05	2.86	3.14
0.050-0.040	1.25	1.05	2.31	1.43
Over 0.050	2.50	1.59
Total.....	100.00	100.00	100.00	100.00

* Data calculated from the registered sedimentation curves.

The degree of dispersion of a carbonate-containing soil suspension, prepared by washing, by the addition of ammonia to the unwashed soil, and by the addition of hydrochloric acid followed by treatment with ammonia

1. *Sample A of the carbonate-containing Dutch soil (1459).*

Table 9 shows the effect of the 3 weeks washing on the degree of dispersion of a soil containing carbonates. The washing of this carbonate-containing soil was strongly dispersing, as the percentage of the fine fractions shows.

2. *Sample B of the carbonate-containing soil (1459).*

The sample B of the carbonate-containing soil was cooked in water, then treated with ammonia to a content of 0.01 normal, 0.1 normal, and 0.5 normal. The results are given in table 10.

The addition of 0.01 normal and 0.1 normal ammonia has been clearly dispersing on the unwashed carbonate-containing soil; as in the case of the car-

carbonate-free soil, the degree of dispersion of the finest fraction decreases in a comparison of the 0.1 normal treatment with the 0.5 normal ammonia. The long continued washing of the carbonate-containing soil appears to have been more active than the addition of ammonia (compare table 9—method 2, with table 10—methods 5, 6, and 7).

That the mere $\frac{1}{4}$ hour cooking is not sufficient to give a uniform homogenous reproducible dispersion in a carbonate-containing soil, is shown by a comparison of sample *A*, table 9, with sample *B*, table 10, treated by the same method

TABLE 9

The influence of washing (for 3 weeks through an unglazed porcelain filter) on the degree of dispersion of the cooked, carbonate-containing Dutch soil 1459*

DIAMETER OF PARTICLES	METHOD 1. SOIL SAMPLE <i>A</i> , COOKED	METHOD 2. SAME SAMPLE, WASHED FOR 3 WEEKS
mm.	per cent	per cent
Under 0.004	17.86	43.17
0.006-0.004	2.38	4.14
0.008-0.006	14.28	
0.010-0.008	1.90	2.00
0.012-0.010	2.38	2.19
0.014-0.012	2.86	2.44
0.016-0.014	2.38	2.00
0.018-0.016	1.90	1.71
0.020-0.018	3.57	0.20
0.030-0.020	7.35	14.10
0.040-0.030	6.50	4.39
0.050-0.040	2.73	2.19
0.060-0.050	3.66	2.44
0.070-0.060	2.20	1.14
0.080-0.070	1.70	1.30
0.100-0.080	2.44	1.46
0.140-0.100	2.20	1.71
0.200-0.140	2.20	2.19
2.000-0.200	19.51	11.46
Total.	100.00	100.23

* Data calculated from the registered sedimentation curves.

1. Sample *A* is more finely divided than sample *B*. Both samples were coagulated by the cooking. Such a coagulation is dependent on a large number of factors which are extraordinarily hard to keep constant. An advantage of the Wiegner apparatus is that, in the study of the changes of a sample, we are independent of the condition at the beginning, as we study the same sample in its changes. Small differences between samples of the same soil cannot be controlled.

3. Sample *C* of the carbonate-containing Dutch soil (1459).

Sample *C* was freed from CaCO_3 with dilute hydrochloric acid in slight

excess. The dissolved substances were removed as fast as possible by washing for 3 weeks through an unglazed porcelain filter. The degree of dispersion was then measured and the further dispersion by 0.1 normal and 0.5 normal ammonia investigated. The results are given in table 11, in which the percentages are based on the carbonate-free soil.

The data show that the treatment with dilute hydrochloric acid and the washing out of the electrolytes gave a somewhat finer dispersion than the

TABLE 10

The influence of the addition of increasing amounts of ammonia on the degree of dispersion of the cooked, carbonate-containing, unwashed Dutch soil (Sample B 1459)*

DIAMETER OF PARTICLES	METHOD 1. SAMPLE B COOKED	METHOD 5 SAMPLE B IN 0.01 N NH ₃	METHOD 6. SAMPLE B IN 0.1 N NH ₃	METHOD 7. SAMPLE B IN 0.5 N NH ₃
mm.	per cent	per cent	per cent	per cent
Under 0.004		21.42	26.92	9.76
0.006-0.004		3.33	5.71	27.50
0.008-0.006		4.76	3.57	3.50
0.010-0.008		2.14	2.38	3.00
0.012-0.010	33.33	2.86	2.38	3.00
0.014-0.012		2.38	2.14	1.75
0.016-0.014		2.14	2.62	3.50
0.018-0.016		2.14	2.62	2.50
0.020-0.018		2.38	2.14	1.38
0.030-0.020	18.33	6.19	5.17	6.10
0.040-0.030	5.47	4.66	5.20	5.13
0.050-0.040	4.47	5.36	4.02	3.86
0.060-0.050	2.90	6.58	4.76	3.57
0.070-0.060	3.09	1.95	2.14	2.14
0.080-0.070	2.38	2.44	2.38	2.38
0.100-0.080	2.38	2.19	3.57	2.62
0.140-0.100	2.38	2.44	2.38	2.38
0.200-0.140	2.14	2.92	2.86	2.38
2.000-0.200	22.60	21.46	16.66	13.81
Total	99.47	99.74	99.62	100.26

* Data calculated from the registered sedimentation curves.

direct treatment with ammonia (compare table 11—method 8, with table 10—methods 5, 6, and 7). The direct washing of the carbonate-containing sample with distilled water acted somewhat more strongly than the acid treatment alone (compare table 11—method 8, with table 9—method 2). The subsequent treatment of the acid extracted sample in 0.1 normal and 0.5 normal ammonia dispersed the soil further than it was possible to reach with any other sample.

RESULTS OF THE INVESTIGATIONS

The results of the investigations are summarized as follows:

1. The action of the preparation of a soil suspension by *shaking*, by *rubbing*, or by *cooking* for the determination of the degree of dispersion is different, depending upon the electrolyte content of the suspension. The cooking of the soil sample is more strongly dispersing in comparison with the other two procedures, if the soil suspension is thoroughly washed and has only a very small content of electrolytes. The particles are then all charged above

TABLE 11

*The degree of dispersion of sample C of the carbonate-containing Dutch soil (1459) treated with dilute HCl for the decomposition of the carbonate, washed and suspended in water, then in 0.1 N and finally 0.5 N ammonia**

DIAMETER OF PARTICLES	METHOD 8. SAMPLE C TREATED WITH HCl AND WASHED	METHOD 9. SAMPLE C IN 0.1 N NH ₃	METHOD 10. SAMPLE C IN 0.5 N NH ₃
mm.	per cent	per cent	per cent
Under 0.004	34 28	47 57	46.66
0.006-0.004	15.71	10.81	16.00
0.008-0.006	5.72	3.24	7 00
0.010-0.008	2 28	5 00	2 73
0.012-0.010	2 86	2 17	2.00
0.014-0.012	2 28	3.24	2.12
0.016-0 014	2 00		2.42
0.018-0 016	2 28		2 73
0.020-0.018	2 30	6 00	2.93
0.030-0.020	5.21	3 86	4 32
0.040-0.030	2 71	6 73	3 02
0.050-0.040	4.73	2.24	6 24
0.060-0.050	2.00	2 22	1 60
0.070-0 060		1.40
0.080-0.070	1 32
0.100-0 080	
0.140-0.100	2.00
0.200-0.140	2.10
2.000-0.200	10 00	4.17	. . .
Total.....	99.78	98.64	99.77

* Data calculated from the registered sedimentation curves.

the critical potential. On the other hand, with a definite electrolyte content, as is present in soil suspensions not washed carefully, the cooking often is more strongly coagulating than the other treatments.

2. The *freezing* of a soil suspension is coagulating on the dispersion if electrolytes are present, as is the case in unwashed soils. If the soil suspensions are thoroughly washed with distilled water, the freezing has no detectable influence on the degree of dispersion of the suspension. The frost action therefore differs according to the electrolyte content of the suspension.

3. The *addition of ammonia*, up to an optimum 0.1 normal concentration, is dispersing on carbonate-free and carbonate-containing soil suspensions which have not been especially

well washed. The washing out of the electrolytes with distilled water in both cases investigated is more strongly dispersing than the direct addition of ammonia to unwashed samples.

Lime-containing and humus-containing samples gave difficultly reproducible results for different samples in the preparation by cooking for $\frac{1}{4}$ hour. Ammonia dispersed these samples further. The most active method for increasing the degree of dispersion was the solution of the carbonate with dilute hydrochloric acid in the cold, a subsequent thorough washing through a filter, and finally the addition of a 0.1 to 0.5 normal ammonia solution.

4. In these investigations, *the apparatus of G. Wiegner with the photographic registering device of H. Gessner*, showed itself suitable for the study of minute changes in the degree of dispersion of one and the same soil sample.⁷

REFERENCES

- (1) BEAM, W. 1911 The mechanical analysis of arid soils. *Cairo Sci. Jour.* 5: 107-119.
- (2) GALLAY, R. 1924 Contribution à l'étude de la coagulation de l'argile. Thesis submitted to l'Ecole Polytechnique, Zurich; *Kolloidchem. Beihelste.* 21: 431-439.
- (3) GESSNER, H. 1922 Der verbesserte Weignersche Schlammapparat. *Mitt. Lebensmitl. Untersuch. u. Hyg., Eidg. Gsndtsamt. (Switz.)* 13: 238-243.
- (4) GESSNER, H. 1926 Der Wiegner'sche Schlammapparat und seine praktische Anwendung. *Kolloid Ztschr.* 38: 115-123.
- (5) NOVAK, V. 1924 Report of the First Commission of the International Conference of Soil Science. Rome.
- (6) ODÉN, SVEN 1925 The size distribution of particles in soils and the experimental methods of obtaining them. *Soil Sci.* 19: 1-35.
- (7) WIEGNER, G. 1918 Ueber eine neue Methode der Schlämmanalyse. *Landw. Vers. Sta.* 91: 41-79.
- (8) WIEGNER, G. 1925 Dispersität und Basenaustausch. *Zsigmondy-Festschrift, Kolloid Ztschr.—Erganzungband.* 36: 341-369; Report of the Second Commission of the Fourth International Conference of Soil Science. Rome.

⁷ The investigations will be continued. The author at this time heartily thanks his co-workers, Dr. R. Gallay, Dr. Aegidius Tschudi, and Dr. R. M. Barnette for their skillful cooperation.

FURTHER STUDIES ON MICHIGAN SOIL PROFILES WITH SPECIAL REFERENCE TO DISPERSED MATERIALS

M. M. McCOOL, A. G. WEIDEMANN, AND G. SCHLUBATIS

Michigan State College Experiment Station

Received for publication July 14, 1926

It has been considered advisable to obtain additional information on the physico-chemical properties of the finer materials that are present in the different horizons of the Michigan soil profiles. Accordingly samples have been taken from the majority of the profiles of the major soil types of the southern and northern groups and brought to the laboratory, where certain studies have been made on them.

The rate of removal of the fine material upon extraction or dispersion with distilled water has been determined as well as the heat of wetting and nitrogen content of the materials dispersed. Furthermore, the amount of colloids present in the various horizons has been estimated by the heat of wetting method. Additional studies are in progress.

RATE OF DISPERSION

The Ontonagon soil profile was selected for use in the study of the rate of dispersion with distilled water and of the differences in the various properties of the different sized particles that remained in suspension over a 24-hour period. About two-pound samples from the A₁, A₂, and B₁ horizons were placed in 2-gallon jars. The dispersion of the fine materials was brought about by filling the jars with distilled water. The contents were vigorously agitated by means of a suction pump constructed of gas pipe and rubber stoppers. Twenty-four hours after agitation, the liquid and suspended materials were siphoned off and run through the Sharpless Super-centrifuge. The same speed of centrifuging was maintained throughout these tests. The liquid was first passed into the bowl through the largest opening and the residue remaining on the celluloid core in the bowl was separated into two portions; namely, the material at the base, designated as no. 1, and that above which formed a much thinner layer, designated as no. 2. The liquid was then passed into the bowl through the medium sized tube and the residue collected and added to the samples of no. 2. Following this treatment the liquid was again run into the bowl through the smallest tube of the separator and the mass collected, designated as no. 3. These processes were repeated twenty-four times, but inasmuch as the solids collected on the last centrifuging were so small in amount, they were grouped as shown in table 1.

It is to be noted from the data presented in table 1 that horizons A₂ and B₁ released more material on the first dispersion than they did in subsequent ones. As a matter of fact, about as much material was dispersed in the first treatment as there was in the remainder. On the other hand a sample collected from A₁ horizon gave up a very small amount of material the first time it was treated with distilled water and under these conditions it would not reach its maximum amount until the third treatment. Similar results have been observed with numerous soil horizons. This may be due to the presence of certain floccu-

TABLE 1
The rate of dispersion of fine material from Ontonagon profile

DISPER- SIONS	A ₁ HORIZON			A ₂ HORIZON			B ₁ HORIZON		
	1	2	3	1	2	3	1	2	3
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	7.5	1	6	385	50	4	330	122	13
2	26.0	3		48	28		57	21	
3	391.0	17		71	10		62	20	
4	100.0	29	3	42	6	1	42	6	2
5	45.0	14		9	4		6	6	
6	100.0	14		30	4		19	3	
7	108.0	18	3	25	9	1	25	5	1
8	7		18	4		19	5	
9	60.0	17		20	7		23	11	
10	37.0	8	1	14	3	1	14	3	1
11	18.0	5		13	3		12	4	
12	25.0	5		9	2		9	3	
13	38.0	10		5	2		11	5	
14	32.0	7		8	2		10	3	
15	23.0	5		9	3		10	4	
16	20.0	5		5	2		12	7	
17	15.0	5		5	1		7	4	
18	13.0	3		5	1		7	4	
19	11.0	2		4	1		
20	11.0	3		3	1		6	3	
21	14.0	3		3	1		5	3	
22	15.0	3		4	1		4	2	
23	9.0		1	1		5	2	

lating substances in the soil which are washed out by the large quantities of distilled water added to the soil.

HEAT OF WETTING

The heat of wetting determinations were made on the various groups of material as well as on that collected from the third dispersion of A₁ horizon and the first dispersion of A₂ and B₁ horizons respectively. The data obtained are given in table 2.

It is clear from these data that the materials that remain in suspension over a 24-hour period vary very widely in their properties; the finer materials, collected at the upper portion of the tube have an appreciably greater heat of wetting those at the base. It is of interest to note here that the materials with the lowest heat of wetting, when redispersed in a large volume of distilled water remained in suspension many days. It is probable that not only the size of the particles or the groups of them but also their specific gravities govern the position that they take in the tube. Several determinations of the specific gravities of some of the materials collected were made with a special type of pycnometer. This type has a bulb at the base and a stem about 10 cm. long with an enlarged upper end into which a ground glass stopper fits. Ligroin was used instead of water in the determinations because the fine materials would not disperse in it, and because there is probably less reactivity between this material and the soil

TABLE 2
Heat developed by each gram of material from Ontonagon soil horizons

	3RD DISPERSION	1ST TO 11TH DISPERSION	6TH TO 11TH DISPERSIONS	12TH TO 23RD DISPERSION
	<i>calories</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>
A ₁ no. 1.	5.36		4.96	5.56
A ₁ no. 2.	9.36		8.21	8.44
A ₁ no. 3.		10.67		
A ₂ no. 1.	4.94		4.88	5.24
A ₂ no. 2.	9.00		7.99	7.60
A ₂ no. 3.		11.73		
B ₁ no. 1.	5.14		5.77	5.98
B ₁ no. 2.	9.53		8.69	8.42
B ₁ no. 3.		11.08		

particles than there is between water and them. The specific gravity of Ontonagon A₁ no. 1 was 2.638; A₁ no. 2, 2.564; B₁ no. 1, 2.795; and B₁ no. 2, 2.761.

Early in the investigations remarkable differences were observed in the color and tenacity of the materials that collected at or near the base of the centrifuge tube and those higher up. Usually the materials at the base proved to be easily pulverized when dry and the remainder much more tenacious and horny-like in nature. The heat of wetting of the former was much lower than that of the latter.

NITROGEN CONTENT OF DISPERSED MATERIALS

The nitrogen content of the above collected solids was determined. It is to be noted from the data in table 3 that the solids obtained by several dispersions were combined in order to obtain a sufficient amount of material for the determinations.

According to the results obtained by the authors there is a tendency for the nitrogen content of the particles obtained from the early and late dispersions, respectively, to be greater than it is of those from the intermediate ones taken from horizons A₁ and A₂, except specimens A₂ no. 2. Other points of interest are; more nitrogen in the materials collected from horizon A₁ no. 1, 2, and 3 than in A₂, no. 1, 2, and 3; and the relatively low amounts of nitrogen in the B₁ samples.

TABLE 3

The nitrogen content of dispersed materials from Ontonagon horizons

HORIZON	DISPERSION	NITROGEN CONTENT
		<i>per cent</i>
A ₁ no. 1	3rd	0.28
A ₁ no. 1	6th to 11th	0.22
A ₁ no. 1	12th to 24th	0.31
A ₁ no. 2	3rd	0.29
A ₁ no. 2	6th to 11th	0.26
A ₁ no. 2	12th to 24th	0.35
A ₁ no. 3	1st to 11th	0.32
A ₂ no. 1	1st	0.10
A ₂ no. 1	6th to 11th	0.09
A ₂ no. 1	12th to 24th	0.21
A ₂ no. 2	1st	0.14
A ₂ no. 2	6th to 11th	0.18
A ₂ no. 2	12th to 24th	0.18
A ₂ no. 3	1st to 11th	0.22
B ₁ no. 1	1st	0.02
B ₁ no. 1	6th to 11th	0.05
B ₁ no. 1	12th to 24th	0.06
B ₁ no. 2	1st	0.06
B ₁ no. 2	6th to 11th	0.09
B ₁ no. 2	12th to 24th	0.06
B ₁ no. 3	1st to 11th	0.07

STUDIES ON MATERIALS COLLECTED BY EVAPORATION

Fine materials were collected from the different horizons of several soil profiles by dispersing them in distilled water. Four hundred grams of the soil were placed in a liter beaker. The beaker was filled with distilled water and the fine material dispersed by agitating with a stirring rod. After 24 hours the liquid was siphoned off and evaporated in an oven. The material was dispersed ten times and each time the liquid was drawn off and added to the dry residue in the evaporator. If after ten dispersions the required amount of fine material had not yet been obtained, a second 400 gm. was taken and the process repeated. In some cases this had to be done several times.

Heat of wetting

The heat of wetting determinations were made on these materials as well as on the original soil, and, on the assumption that the heat of wetting is all due

TABLE 4
Heat of wetting of soils and dispersed materials

SOIL TYPE	HORIZON	AMOUNT OF HEAT FROM EACH GRAM OF MATERIAL		COLLOIDS IN SOIL
		Soil	Colloids	
		<i>calories</i>	<i>calories</i>	<i>per cent</i>
Fox sandy loam.....	A	1.32	6.53	20.21
	B ₁	2.50	9.04	27.65
	B ₂	1.82	8.84	20.58
Hillsdale heavy sandy loam	A	0.93	5.91	15.73
	B ₁	1.19	6.39	18.70
	B ₂	1.24	6.07	10.42
Napawee silt loam.	A	1.96	5.35	35.51
	B	2.93	7.13	41.09
	C	1.99	3.92	50.76
Kewawee loam	A	1.38	6.22	22.18
	B		7.33	
	C	1.72	4.61	37.31
Brookston silt loam	A	7.98
	B	..	8.53	..
	C	..	5.53
Strong light sandy loam	A ₁	0.42	8.70	5.18
	B ₁	2.53	10.94	23.12
	B ₂	1.11	9.97	11.13
Chippewa light sandy loam	A ₁	0.72		
	B ₁	2.66	19.93	13.34
	B ₂	1.66	14.37	11.55
Onaway loam	A ₁	0.49	4.20	11.66
	A ₂	1.02	4.85	21.03
	B ₁	2.41	6.68	36.07
Stalwart light sandy loam	A ₁	1.12	4.98	22.49
	A ₂	2.19	8.55	25.61
	B ₁	1.62	7.71	21.01
Ontonagon silt loam	A ₁	2.96	4.88	60.65
	A ₂	3.21	5.57	57.63
	B ₁	4.63	6.61	70.04

TABLE 5

Nitrogen in soils and fine materials

SOIL TYPE	HORIZON	NITROGEN IN SOIL	NITROGEN IN FINE MATERIAL
		<i>per cent</i>	<i>per cent</i>
Fox	A	0.081	0.16
	B	0.048	0.09
	B ₂	0.036	0.08
Hillsdale	A	0.039	0.16
	B ₁	0.010	0.10
	B ₂	0.048	0.14
Napawee	A	0.112	0.30
	B	0.084	0.12
	C	0.070	0.09
Kewanee	A	0.050	0.16
	B	0	0.03
	C	0.045	0.07
Miami	A	0.224	0.38
	B	0.092	0.17
	D	0.070	0.10
Brookston	A	0.112	0.30
	B	0.110	0.25
	C	0.030	0.10
Strong's	A ₁	0.106	0.95
	B ₁	0.143	1.21
	B ₂	0.098	1.00
Chippewa	A ₁	0.062	1.04
	B ₁	0.092	1.12
	B ₂	0.143	1.24
Onaway	A ₁	0.095	0.24
	A ₂	0.050	0.19
	B ₁	0.059	0.21
	C	0.035	0.06
Stalwart	A ₁	0.070	0.28
	A ₂	0.087	0.20
	B ₁	0.062	0.16
Ontonagon	A ₁	0.148	0.29
	A ₂	0.081	0.13
	B ₁	0.050	0.07

to the colloids and that the particles so extracted are representative of the mass, the percentage of colloids in the soil was estimated by the use of the formula

$$\frac{\text{Heat of Wetting of Soil}}{\text{Heat of Wetting of Colloids}} \times 100.$$

These results are given in table 4.

The heat of wetting values in table 4 are interesting in that the materials extracted from the northern Michigan soils, Strong and Chippewa, are remarkably active; especially is this true of the B horizons. The B horizons of the southern Michigan soils are also very active as measured by the heat evolved when they are brought into contact with water. As judged by the touch method, the amounts of colloids present in the Stalwart and Chippewa horizons are surprisingly high, otherwise the results are about as one would predict and similar to those previously reported.

Nitrogen content

The nitrogen content of the original samples and of the extracted materials has been determined and is shown in table 5. The results obtained from the studies on the various horizons are similar to those previously reported (10, 11). Notably, the coarser textured, strongly podsolized northern types or Strong, Chippewa, and Stalwart have high percentage of nitrogen in the B layers, whereas the finer textured ones, or the Ontonagon and Onaway as sampled, carry more of the element in the A horizon, the exception being A₁ of the latter type. The nitrogen content of the fine materials extracted from the northern soils, Chippewa and Strong, is strikingly high and the indications are that the majority of it in these types exists in the colloidal state. The probabilities are that the colloids for the most part are in the sol state.

REFERENCES

- (1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.* 28: 927-935.
- (2) ANDERSON, M. S., AND MATTSO, S. 1925 The relation between properties and chemical composition of soil colloids. *Science* 62: 114-115.
- (3) BOUYOUCOS, G. J. 1924 Heat of wetting as a new means of estimating the colloidal material in soils. *Science* 60 (1553): 320.
- (4) BOUYOUCOS, G. J. 1925 Chief factors which influence the heat of wetting of soil colloids. *Soil Sci.* 19: 477-482.
- (5) BRADFIELD, R. 1924 Importance of hydrogen-ion concentration control in physico-chemical studies of heavy soils. *Soil Sci.* 17: 411-422.
- (6) BRADFIELD, R. 1925 Chemical nature of colloidal clay. *Jour. Amer. Soc. Agron.* 17: 253-270.
- (7) EDINGTON, G., AND ADAMS, J. R. 1925 Distribution of nitrogen in the podsol profile. *Soil Sci.* 20: 177-179.
- (8) GORDON, N. E., AND STARKEY, E. B. 1922. Influence of soil colloids on availability of salts. *Soil Sci.* 14: 1-7.

- (9) JOSEPH, A. F. 1925 Clay as soil colloids. *Soil Sci.* 20: 89-94.
- (10) MCCOOL, M. M., VEATCH, J. O., AND SPURWAY, C. H. 1923 Soil profile studies in Michigan. *Soil Sci.* 16: 95-106.
- (11) MCCOOL, M. M., AND WHEETING, L. C. 1924 The influence of the removal of colloids on some soil properties. *Soil Sci.* 18: 99-102.
- (12) ROBINSON, W. O., AND HOLMES, R. S. 1924 Composition of soil colloids. U. S. Dept. Agr. Bul. 1311.
- (13) WHITNEY, M. 1921 The origin of soil colloids and the reason for the existence of this state of matters. *Science* 54 (1409): 653-656.

FACTORS AFFECTING THE HYDROGEN-ION CONCENTRATION OF SOILS¹

L. D. BAVER²

Ohio Agricultural Experiment Station

Received for publication July 20, 1926

The hydrogen-ion concentration is perhaps the easiest and quickest method of studying the reaction of soils. It is used extensively as an expression of the acidity or alkalinity of a soil. The determination of the hydrogen-ion concentration in the laboratory and the use of the values thus obtained as expressions of the acidity in the field, however, involve the consideration of several factors: In the first place, does the hydrogen-ion concentration of a soil that has been prepared for the usual laboratory analyses give a true expression of the acidity of the soil in the field? Secondly, is the reaction of the soil in the field constant or variable?

It is the purpose of this paper to report certain studies which have been made of the factors that might influence the hydrogen-ion concentration of soils. Consideration was given to the effect of air-drying and grinding a soil on its reaction; to the periodical variations in the hydrogen-ion concentration of soils; to the effect of fertilizer treatments on soil acidity; and to the effect of surface treatment on the reaction of the subsurface layers. The results were obtained by the use of the quinhydrone electrode (4).

EXPERIMENTAL

Most of the experimental work in this study was carried out with the Wooster silt loam, which is the typical soil at the main station farm. It is a glacial sandstone and shale soil, acid throughout its entire profile. The surface and subsurface soil is a brown silt loam. Below 14 inches, the subsoil is a yellowish brown, light, silty clay loam. A further description of this soil together with its reaction profile is shown in figure 1.

The effect of air-drying a soil

Air-drying a soil often produces a change in its hydrogen-ion concentration. It is questionable, however, if the change that is brought about by air-drying

¹ Published with the permission of the Director of the Ohio Agricultural Experiment Station.

² The author expresses his appreciation of the kindly suggestions and criticisms of Professors G. W. Conrey and R. M. Salter.

is significant enough to warrant the conclusion that only fresh, moist samples should be used for determining the hydrogen-ion concentration of soils. Burgess (10) found that air-drying acid soils had little or no effect upon the pH values but that drying alkaline soils caused them to become somewhat less alkaline. With the latter he observed a decrease in alkalinity of 0.4 pH. Rost and Fieger (28) report a tendency toward increased acidity upon air-drying both acid and alkaline soils. Some became slightly more acid, others

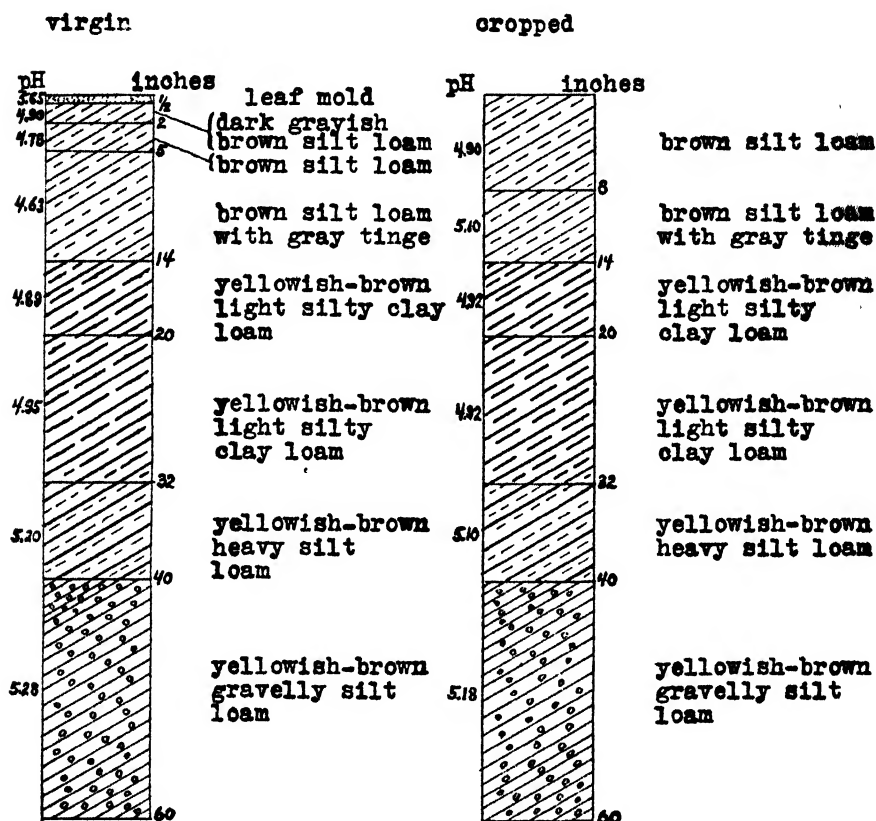


FIG. 1. DESCRIPTION AND REACTION PROFILE OF WOOSTER SILT LOAM

slightly less acid. They conclude that the only reliable indication of conditions existing in the field is obtained by using fresh samples for making hydrogen-ion determinations. Gardner and Brown (16) could not find a uniform effect of air-drying upon the lime requirement of a soil as determined by the Veitch method.

The results obtained in the present study shown in table 1 indicate that air-drying an acid soil does not significantly affect its reaction. However, there is a considerable decrease in alkalinity with the alkaline soils. With the sub-

soils, air-drying has caused a marked increase in acidity, which increases with depth. The amount of lime applied to the soil seems to have some influence on the change in reaction. Sample L-2 receives 2 tons of ground limestone to the acre; L-12, 4 tons; and L-20, 8 tons to the acre. There is a decrease in alkalinity upon air-drying these soils of 0.32, 0.23, and 0.01 pH, respectively. Samples L-12 and L-20 had practically the same reaction in the fresh condition. Thus, it would appear that the lime content of the soil might have some effect on the changes in the hydrogen-ion concentration of the soil as brought about by air-drying.

It is evident from the data in table 1 that the use of fresh samples for hydrogen-ion determinations gives a closer expression of the reaction of the soil as it occurs in the field than do the air-dried samples. With the acid surface soils, however, there was no appreciable change in reaction. It seems, therefore, that for practical purposes the hydrogen-ion concentration of an air-dried soil would give a sufficiently accurate expression of its reaction, but that fresh samples should be used for making refined reaction studies.

The effect of grinding

Grinding a soil may have a marked effect on its reaction. Brown and Johnson (8) found that grinding Iowa soils through an 80-mesh sieve considerably reduced their lime requirement, determined by the Veitch method, and frequently caused the reaction to become basic. Cook (13), on the other hand, observed that grinding sandy soils from New Jersey increased their acidity as determined by the Veitch method. Cook attributes the difference in the results of Brown and Johnson to a difference in soil type. Table 2 shows the effect of grinding a soil through a 100-mesh sieve on its hydrogen-ion concentration. Grinding the acid samples of the Wooster silt loam did not materially affect their reaction, but grinding the alkaline soils decreased their alkalinity. This was probably due to the exposing of surfaces that had not been affected by the additions of lime. The Brookston and Miami clays, glacial limestone soils, exhibit a decrease in acidity upon grinding. The Dunkirk fine sand, a glacial lake sand, shows a large decrease in acidity after it is ground.

This same effect was observed by Brown and Johnson (8) of Iowa. They attributed this reduction in acidity upon grinding to a solution of the minerals in the soil. Cook (13) concludes that perhaps the soils of Iowa are not "sands" in the purely chemical sense, but consist of particles having a basic internal constitution, the surface of which may have become acid on exposure. It appears that these reductions in acidity are probably due to the exposing of basic materials that are included in the soil particles. At any rate the results indicate that grinding a soil changes its reaction to such an extent that only unground samples should be used for making hydrogen-ion determinations.

TABLE 1
The hydrogen-ion concentration of the soil as influenced by air-drying

PLOT	DEPTH	MOIST SAMPLE	AIR-DRIED SAMPLE	CHANGE IN H-ION CONCENTRATION
	<i>inches</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
1-E*	0-7	5.02	4.94	-0.08
2-E	0-7	4.85	4.83	-0.02
3-E	0-7	4.81	4.75	-0.05
4-E	0-7	4.76	4.75	-0.01
5-E	0-7	4.85	4.76	-0.09
6-E	0-7	4.84	4.90	+0.06
7-E	0-7	4.75	4.70	-0.05
24-E	0-7	4.41	4.47	+0.06
11	0-7	4.85	4.89	+0.04
18	0-7	5.03	5.09	+0.06
22	0-7	5.06	5.00	-0.06
24	0-7	4.56	4.56	0.00
1-W	0-7	8.14	7.81	-0.32
2-W	0-7	7.68	7.50	-0.18
3-W	0-7	7.77	7.45	-0.32
4-W	0-7	7.77	7.45	-0.32
5-W	0-7	7.86	7.56	-0.30
6-W	0-7	7.72	7.43	-0.29
7-W	0-7	7.71	7.39	-0.32
8-W	0-7	7.71	7.33	-0.38
9-W	0-7	7.71	7.52	-0.19
10-W	0-7	7.77	7.60	-0.17
11-W	0-7	7.86	7.54	-0.32
12-W	0-7	7.76	7.64	-0.12
L-4	0-7	5.41	5.31	-0.10
L-4	7-14	5.60	5.12	-0.48
L-4	14-21	5.35	4.81	-0.54
L-4	21-28	5.32	4.69	-0.63
L-2	0-7	6.88	6.47	-0.41
L-2	7-14	5.54	5.22	-0.32
L-2	14-21	5.33	4.81	-0.52
L-2	21-28	5.29	4.79	-0.59
L-12	0-7	7.75	7.52	-0.23
L-12	7-14	6.25	5.86	-0.39
L-12	14-21	5.59	5.20	-0.39
L-12	21-28	5.49	4.95	-0.54
L-20	0-7	7.73	7.72	-0.01
L-20	7-14	6.59	6.23	-0.36
L-20	14-21	5.74	5.36	-0.38
L-20	21-28	5.51	5.10	-0.41

* Plots E and W are from the 5-year rotation, east and west ends.
 Plots L are from the supplemental lime tests.

Periodical variations in reaction

The time of sampling appears to be a significant factor in studying the hydrogen-ion concentration of soils. Burd and Martin (9) found changes varying from 0.2 to 0.6 pH from April to September. Burgess (11) reports similar variations from May to September, the acidity being greater during July and August. Erdman (15) shows fluctuations of 0.35 pH from March until August. The acid soils exhibited greater acidity at the close of this period, but there was no consistency in the variation of the alkaline soils. Gardner and Brown (16) in periodically studying the lime requirement of fertility plots report variations from 0 to 1040 pounds to the acre from June 15

TABLE 2
The effect of grinding a soil on its reaction

SOIL		DEGREE OF FINENESS		CHANGE IN H-ION CONCENTRATION
Number	Type	2 mm.	100-mesh	
		pH	pH	pH
1-E	Wooster silt loam	4.94	4.96	+0.02
2-E		4.83	4.81	-0.02
3-E		4.75	4.90	+0.15
4-E		4.75	4.90	+0.15
5-E		4.76	4.76	0.00
1-W		7.81	7.56	-0.25
2-W		7.50	7.18	-0.32
3-W		7.45	7.12	-0.33
4-W		7.45	7.14	-0.31
5-W		7.56	7.27	-0.29
6-A	Brookston clay	6.24	6.46	+0.22
7-A	Miami clay	5.11	5.35	+0.24
85-A	Dunkirk fine sand	5.87	6.87	+1.00
88-A		5.55	6.84	+1.29

to August 15. The acid plots showed a higher lime requirement in August than in June. Kelley (20) observed changes of 1.0 pH during the entire year, with a continual increase in acidity from July to October. Kurz (21) noticed deviations of 1.0 pH during the year. Lipman and his associates (22) show fluctuations of 0.7 pH from May to November. Mather (24) reports variations of 0.7 pH from May to July with an increase in acidity in July.

In a preliminary study of the reaction of several of the 5-year rotation fertility plots, samples that were taken in the late summer or fall were found to be more acid than those taken in the spring (table 3). This led to a periodical study of the hydrogen-ion concentration of the soil. Four different samples were taken periodically during the growing season of 1925. Samples A and

TABLE 3

Periodical variation in the hydrogen-ion concentration of several fertility plots of Section D, 5-year rotation

PLOT	DATE OF SAMPLING					
	1894	1923 August 1	1924 April 17	1924 June 30	1925 April 21	1925 September 25
	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
2	5.05	4.75	4.97	4.83
3	5.07	5.04	4.64	4.93	4.75
5	5.14	4.55	4.91	4.76
11	4.88	5.06	4.65	4.85	4.74
18	4.96	5.04	5.24	4.68	5.03	4.92
22	4.90	4.98	5.08	4.80	5.06	4.88
24	4.97	4.73	4.90	4.39	4.56	4.47

TABLE 4

The relation of rainfall to variations in the hydrogen-ion concentration of the soil

DATE	RAINFALL		SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
		<i>inches</i> [*]	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>
April 29	Before	0.47	7.10	5.83
May 2	After		7.22	5.61
May 2	Before	0.70	7.22	5.61
May 8	After		7.17	5.46
May 8	Before	0.73	7.17	5.46
May 11	After		6.80	5.52
May 15	Before	0.24	7.35	5.55
May 18	After		7.08	5.40
May 21	Before	0.65	7.05	5.65
May 26	After		7.08	5.40
July 13	After	2.65	6.98	5.33	6.10	4.92
July 17	Before	0.31	7.25	5.30	6.30	4.88
July 24	After		7.12	5.11	6.31	4.86
Sept. 16	After	2.49	7.15	5.04	6.52	4.71
Sept 21	Before	0.02	7.40	5.00	6.70	4.79
Sept. 23	After		7.33	5.04	6.57	4.81

B were sod plots having alkaline and acid reactions, respectively. Samples C and D were taken from cultivated plots. These were chosen to study the relation, if any, between cultivation and the periodic change in the soil reaction. At the time of sampling, a record was kept of the moisture content of the soil, the rainfall, the relative humidity, and the temperature in order to observe any correlation between these factors and the changes in the hydro-

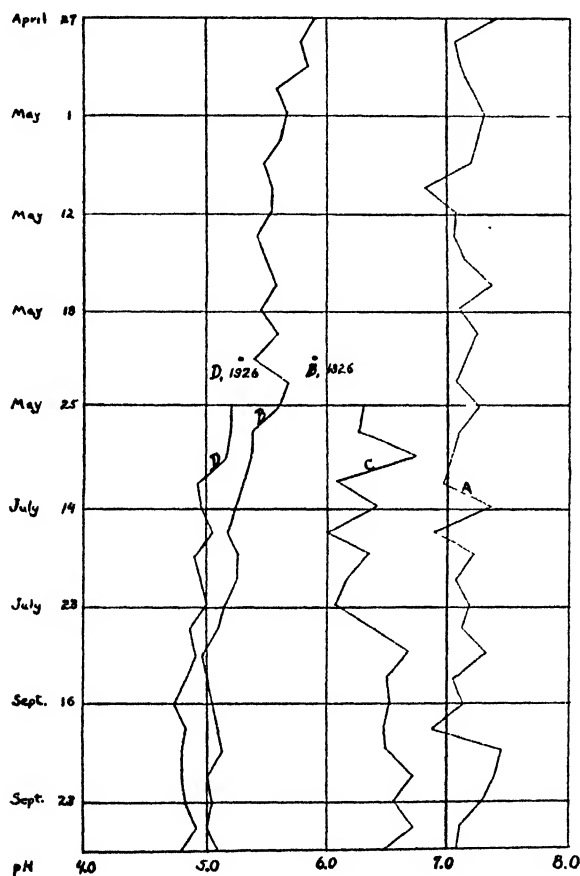


FIG. 2. VARIATIONS IN SOIL REACTION ON FOUR PLOTS FROM APRIL 27 TO SEPTEMBER 23

gen-ion concentration. All hydrogen-ion determinations were made on the air-dried samples.

The results in table 4 show variations in the pH values of samples A, B, C, and D of 0.60, 0.92, 0.71, and 0.54, respectively. The acid samples, B and D, exhibit a continual increase in their hydrogen-ion concentrations from May to September. These results are in close agreement with those reported by Burgess (11), Erdman (15), Gardner and Brown (16), Kelley (19), and Mather/

(23), who observed an increase in acidity from the spring months to the late summer and fall. The alkaline plots show no consistent variations during this period. A graphic representation of the data shows these changes more clearly (fig. 2).

TABLE 5
Periodical variations in the reaction of a soil

DATE	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
	pH	pH	pH	pH
April 27, 1925	7.40	5.89
28	7.06	5.78	.	..
29	7.10	5.83
30	7.18	5.56
May 1	7.27	5.64
2	7.22	5.61	.	..
8	7.17	5.46	.	..
11	6.80	5.52	.	..
12	7.05	5.52
13	7.04	5.39	.	.
14	7.11	5.44
15	7.35	5.55
18	7.08	5.40
19	7.24	5.55
20	7.15	5.35
21	7.05	5.65	..	.
25	7.25	5.57	6.31	5.25
26	7.08	5.40	6.27	5.24
27	7.01	5.39	6.71	5.20
July 13	6.98	5.33	6.10	4.92
14	7.39	5.28	6.40	4.94
15	6.90	5.19	6.00	5.06
17	7.25	5.30	6.30	4.88
22	7.07	5.29	6.16	..
23	7.21	5.20	6.10	5.00
24	7.12	5.11	6.31	4.86
Sept. 14	7.36	4.97	6.63	4.90
15	7.02	5.00	6.50	4.80
16	7.15	5.04	6.52	4.71
17	6.83	5.10	6.47	4.82
18	7.46	5.11	6.50	4.80
21	7.40	5.00	6.70	4.79
23	7.33	5.04	6.57	4.81
24	7.12	5.00	6.71	4.90
29	7.10	5.09	6.45	4.77
May 20, 1926	5.85	...	5.25

There appears to be no definite correlation of moisture content, relative humidity, and temperature with the changes in the soil reaction. Rainfall seems to have some influence on the variations in the pH values (table 4). The acidity of the soil, observed before and after a precipitation of rain, in-

creased after the rain 12 times in 18 observations. Kelley (20) also observed an increase in the hydrogen-ion concentration following a rain.

The time of year appears to be an important factor, in the case of the acid soils especially. There is a consistent increase in acidity from the spring to the fall with a return to approximately the original acidity the next spring. Two probable explanations might be given for this phenomenon: First, it may be due to the dehydration of the hydrated colloidal silicates with a decrease in buffer action during the hot and dry summer months. The rainfall and moisture conditions of the winter and spring saturate the soil with water and the colloidal silicates resume the hydrated state, thereby causing the acidity to return to about the same value each spring. Or, in the second place, there may be an accumulation of salts in the soil during the summer which would tend toward increased acidity. During the winter and spring these salts would be leached away, producing a reduction in acidity. This recurrence of acidity was also observed by Kelley (20). It is shown in tables 3 and 5 and figure 2.

The foregoing results show the importance of the seasonal factor in studying the hydrogen-ion concentration of soils. Therefore, to say that a given soil has a definite pH value implies that there may be a variation of more than 0.2 in pH above or below the assigned value from time to time. The determination of the hydrogen-ion concentration of a field soil and its expression in terms of pH to the second decimal place scarcely seems logical if one considers these variations.

The effect of fertilizer treatment

There has been considerable discussion as to the effect of fertilizer treatments on the acidity of the soil. Most of the investigators, however, conclude that with the exception of ammonium sulfate the various fertilizers exert no appreciable influence on the reaction of the soil. Allison and Cook (1), Blair and Prince (6), Brioux (7), Crowther (14), Gardner and Brown (16), Lipman and his associates (23), Morse (25), Plummer (27), Stephenson (30), and Veitch (31) observed that ammonium sulfate increased the acidity of the soil. Sodium nitrate had a tendency to reduce it. Ames and Schollenberger (2), in studying the loss of calcium carbonate from the soil, could not find any consistent variations which could be attributed to soil treatment with the exception of the ammonium sulfate plot which showed a complete loss of the carbonate. Hall (17) reported the largest losses of calcium carbonate from the ammonium sulfate plot and the smallest from sodium nitrate in Broadbalk and Hoos Fields at Rothamsted. Morse (26) observed that twice as much calcium carbonate was leached away in the drainage waters from the ammonium sulfate treatment as from sodium nitrate.

In regard to acid phosphate, Skinner and Beattie (29) claim that it causes an increase in the acidity of the soil. On the other hand, Ames and Schollenberger (2), Brioux (7), Crowther (14), Karraker (19), Lipman and associates

(23), Morse (25), and Plummer (27) found no material effect of acid phosphate on soil acidity. Bauer and Haas (3), Bear and Salter (5), Conner (12), and Veitch (31) conclude that acid phosphate tends somewhat to reduce the acidity of the soil.

Manures do not materially affect the soil reaction as reported by Ames and Schollenberger (2), Howard (18), Karraker (19), and Stephenson (30). Crowther (14), however, observed a reduction of about 0.5 pH in the soil at Woburn with the use of barnyard manure.

TABLE 6

The effect of 30 years' fertilizing and cropping on the hydrogen-ion concentration of the Wooster silt loam

PLOT	SECTION D*			
	Treatment	East half† 1894	East half September 1925	West half† September 1925
		pH	pH	pH
2	P	..	4.83	7.50
3	K	5.07	4.75	7.45
4	None	5.14	4.75	7.45
5	N	..	4.76	7.56
11	N, P, K	4.88	4.74	7.54
18	Manure, 16 T	4.96	4.92	7.55
19	None	5.03	4.84	7.41
20	Manure 8 T.	5.16	4.86	7.60
24	N in $(\text{NH}_4)_2\text{SO}_4$	4.97	4.47	7.16
25	None	5.08	4.77	7.59
26	P in bonemeal	5.19	4.97	7.50
29	P in basic slag	5.19	4.93	7.68

* For a more complete outline of the fertilizer treatment of these plots, see Ohio Agr. Exp. Sta. Bul. 381.

† The time of the year when these samples were taken is not known.

‡ 32,654 pounds of calcium carbonate since 1894.

Erdman (15) observed that gypsum in excess of 1000 to 2000 pounds to the acre increased the acidity of the soil, but when added in smaller amounts it had practically no effect.

The 5-year rotation fertility plots at the Ohio Station afford an excellent opportunity for studying the effects of different fertilizer treatments on soil acidity. In this experiment corn, oats, wheat, clover, and timothy are grown in succession on five sections of land, containing 30 one-tenth acre plots each. The experiment was started in 1894. At this time the deficiency of the soil

in lime was not noticed, but the failing of the clover crops soon showed the necessity of liming. In 1900 the experiment of liming half of each plot was started. Since then the entire west half of section D has received ground limestone amounting to about 16 tons, applied to the corn. This has been added at the rate of about 2 tons to each acre. Table 6 gives the pH values of plots receiving standard fertilizer treatments, together with the hydrogen-ion concentration of some of the samples taken in 1894. A complete report of the reaction of all of the fertility plots will appear later.

The unfertilized plots have an average pH value of 4.76. It is evident from the results in table 6 that mineral manures, with the exception of ammonium sulfate, have caused no significant change in the reaction of this soil. The ammonium sulfate plot (plot 24) is the most acid, having a pH value of 4.47 at this time. The results indicate that acid phosphate, manure, bone-meal, and basic slag have a slight tendency to decrease the acidity. The application of lime has corrected the acidity in all cases. In most of the plots a slight alkalinity has developed. The use of lime for neutralizing the acids produced from ammonium sulfate is readily seen in plot 24 which after liming has a pH value of 7.16.

From the difference in the results observed in 1894 and 1925, it might be inferred that there has been considerable increase in acidity in some of the plots, but this conclusion cannot be safely drawn. In the first place, the 1894 samples were taken with a spade and perhaps are not very representative of the true reaction of the soil at that time. The large variation in a few of the samples tends to justify this statement. Secondly, the exact time of sampling in 1894 is not known. This is an important factor as previous results have shown. The results in table 3 show that the difference between the 1894 and 1925 samples are not as great as the seasonal variation. Thus, it appears that there has been no significant change in the reaction of the untreated plots during the last 30 years. Since it appears that agricultural practices, without the use of fertilizers and soil amendments, in the last three decades have not caused an increase in soil acidity, it seems that an equilibrium point in the hydrogen-ion concentration of the Wooster silt loam perhaps has been reached. In this case, the process of leaching and removal of bases from the soil has either reached a minimum or some other factor is maintaining the present hydrogen-ion concentration of the soil. Before any conclusions can be definitely established in regard to this question, further studies must be made. At this time, however, the fact must not be overlooked that 30 years is a short time when compared with the total length of time that the soil has been leaching.

The effect of surface treatment on the reaction of the subsurface layers

It is of interest to determine the extent to which a change of reaction in the surface layer affects the lower depths of the soil. A series of plots from the supplemental lime tests, which were started in 1911, were sampled at suc-

cessive depths of 7 inches down to 28 inches. These plots have received applications of ground limestone ranging from 0 to 8 tons to the acre, applied to the corn in a 4-year rotation of corn, oats, wheat, and clover. The hydrogen-ion concentrations of the different depths are reported in table 7. In addition to these results, the pH values of the surface and subsoil of the lime

TABLE 7
The effect of surface treatment on the reaction of the subsurface layers

PLOT*	LIME TREATMENTS		HYDROGEN-ION CONCENTRATION AT DIFFERENT DEPTHS			
	Kind of lime applied in pounds per acre	Total amount applied per plot in terms of CaCO ₃	0 to 7 inches	7 to 14 inches	14 to 21 inches	21 to 28 inches
		pounds	pH	pH	pH	pH
L-4	None	None	5.31	5.12	4.81	4.69
L-2	4,000, ground limestone + manure	400	6.47	5.22	4.81	4.79
L-12	8,000, ground limestone + manure	800	7.52	5.86	5.20	4.95
L-20	16,000, ground limestone + manure	1,600	7.72	6.23	5.36	5.10
				7 to 20 inches		
LF-1	None	None	5.58	5.16		
LF-2	500, quicklime + manure	310	6.44	5.29		
LF-3	1,000, quicklime + manure	620	7.13	5.43		
LF-5	2,000, quicklime + manure	1,250	7.94	6.64		
LF-6	1,780, ground limestone + manure	620	7.05	5.71		
LF-7	None	None	5.58	5.43		
LF-8	1,780, air-slaked lime + manure	840	7.40	5.77		
LF-9	1,320, hydrated lime + manure	620	7.27	5.95		
LF-14	1,000, quicklime	620	7.12	5.74		
LF-15	1,780, limestone	620	6.59	5.52		
LF-16	None	None	5.36	5.31		
LF-17	1,000, quicklime + acid phos., KCl	620	7.00	5.45		
LF-18	1,000, quicklime + floats, KCl	620	7.03	5.53		

* Plots L from the supplemental lime tests.

Plots LF from the lime and floats test. For a complete description of the plots see Ohio Agr. Exp. Sta. Bul. 381.

plots from the lime and floats experiment are given. The total amount of lime, in terms of calcium carbonate, that each plot has received since the beginning of the experiment is recorded in the third column of the table. The effect of the surface treatment on the lower depths is shown more definitely in figure 3.

As these results clearly show, the 2 ton application of lime (plot L-2), has had little effect below 7 inches. With the 4 (plot L-12) and 8 (plot L-20) ton applications there has been a marked decrease in acidity in the second depth, with a gradual reduction in the hydrogen-ion concentration to 28 inches. The 8 ton addition has produced the largest reduction. In the lime

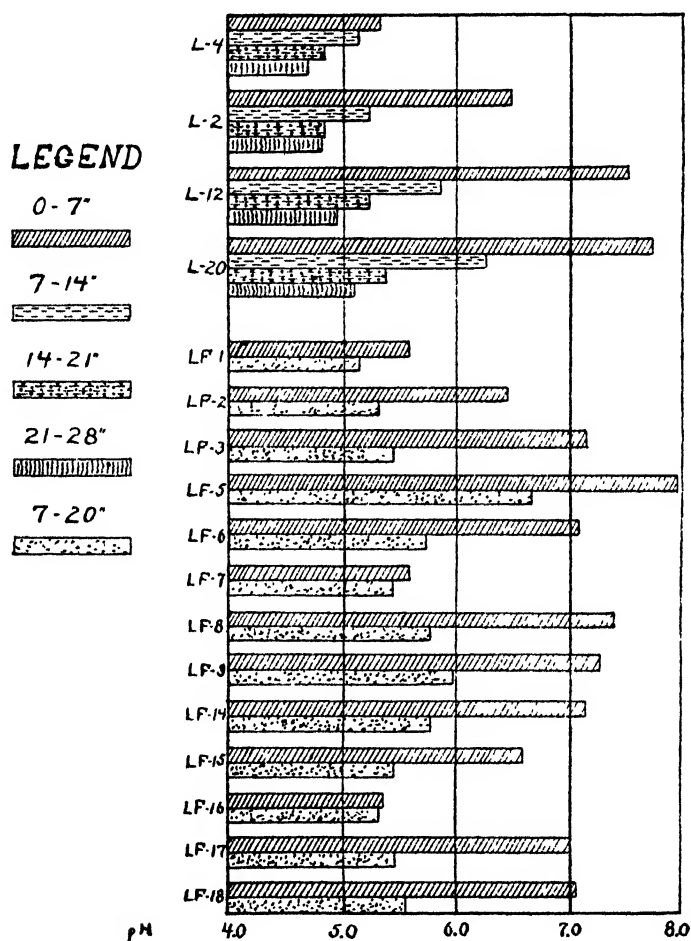


FIG. 3. EFFECT OF SURFACE TREATMENT ON SUBSURFACE LAYERS

and floats plots, the effect of lime is detected in all the subsoils with the exception of the 500 pound application of quicklime. Plot LF-5, with 2000 pounds of calcium oxide, has produced the largest reduction in the acidity of the subsurface layer.

It is logical to assume that the effect of the surface treatment on the lower depths will be more pronounced as the experiments progress. The buffer

action of soils and the relatively slow movement of the soil water are two of the determining factors in the rate of change in the reaction of the subsoil layers. The subsoil reaction, especially to a depth of 20 inches, should be a factor in determining the efficiency of liming. It is improbable that a calcium carbonate reserve in the soil will be maintained unless the acidity of the subsoil to at least 20 inches is corrected.

SUMMARY

In this paper are presented the results of an investigation of some of the probable factors that affect the hydrogen-ion concentration of soils.

The Wooster silt loam, the soil used in these investigations, is a glacial sandstone and shale soil having a decidedly acid reaction profile.

Air-drying the acid surface soil did not materially affect its hydrogen-ion concentration. The alkaline soils decreased in alkalinity.

Subsoil samples showed a large increase in acidity when they were air-dried.

The amount of lime in the soil appears to have some effect upon the changes in the reaction of a soil when air-dried.

Grinding acid samples of the Wooster silt loam did not appreciably change their reaction. Alkaline samples decreased in alkalinity, probably because of the exposing of surfaces that had not been affected by the additions of lime.

Grinding the Brookston and Miami clay and the Dunkirk fine sand decreased their acidity considerably. This decrease is probably due to the exposing of basic materials upon grinding.

The hydrogen-ion concentration of a soil varies throughout the year. Alkaline soils showed variations in acidity ranging from 0.6 to 0.7 pH from May to September.

Acid soils varied as much as 0.92 pH during this period. There was a continual increase in acidity from May to September, with the hydrogen-ion concentration returning to approximately the same value each spring. This phenomenon might be due to the dehydration of the hydrated colloidal silicates or to the accumulation of soluble salts during the summer months.

Rainfall and the season seem to be two important factors affecting these variations in the soil reaction.

Fertilizer treatments do not influence the hydrogen-ion concentration of soils to any considerable extent, with the exception of ammonium sulfate which causes an increase in acidity; although acid phosphate, sodium nitrate, bonemeal, basic slag, and manure tend somewhat to reduce it.

Applications of lime have corrected the acid nature of the soil, producing a slightly alkaline reaction.

There appears to have been no significant change in the hydrogen-ion concentration of the Wooster silt loam in the last 30 years.

From these results, the assumption is made that an equilibrium point may have been reached in the reaction of this soil, whereby, under ordinary conditions, the hydrogen-ion concentration will not show further change.

The surface treatment of a soil influences the reaction of the lower depths. Over a period of 11 years, 4 and 8 tons of ground limestone, added every 4 years in the rotation, have affected the reaction of the subsoil as deep as 28 inches. The effect of 2 tons per acre can be seen only in the surface layer.

The acidity of the subsoil of plot 5 of the lime and floats experiment, receiving 2000 pounds of calcium oxide to the acre every 3 years for 20 years, has been reduced almost to neutrality, having a pH of 6.64 at a depth of 20 inches.

CONCLUSIONS

A study of some of the factors that may affect the hydrogenion concentrations of soils leads to the following conclusions:

1. Fresh, moist samples should be used for making detailed reaction studies. Air-dried samples seem to give sufficiently accurate results for any ordinary purposes.
2. Hydrogen-ion concentration determinations should be made on unground soil samples. Grinding affects the reaction of a soil to such an extent that the results may be misleading.
3. The periodical variation in the pH values of soils should be considered in all hydrogen-ion concentration measurements. The expression of the reaction of a field soil in terms of pH to the second decimal place does not appear logical in view of the variability of the pH value.
4. The hydrogen-ion concentration of the soil is not materially affected by fertilizer treatments, with the exception of ammonium sulfate. The increase in acidity from the use of ammonium sulfate in ordinary amounts, however, is not significant enough to diminish its value as a commercial fertilizer.
5. The hydrogen-ion concentration of the subsoil is influenced by the treatment of the surface soil. The reaction of the subsurface layers should be a factor in determining the efficiency of liming. The removal of subsoil acidity to at least 20 inches is necessary to maintain a calcium carbonate reserve in the soil.

REFERENCES

- (1) ALLISON, F. E., AND COOK, R. C. 1916 The effect of ammonium sulfate on soil acidity. *Soil Sci.* 3: 507-512.
- (2) AMES, J. W., AND SCHOLLENBERGER, C. J. 1916 Liming and lime requirement of soil. Ohio Agr. Exp. Sta. Bul. 306.
- (3) BAUER, F. C., AND HAAS, A. R. C. 1922 The effect of lime, leaching, form of phosphate and nitrogen salt on the plant and soil acidity and the relation of these to the feeding power of the plant. *Soil Sci.* 13: 461-479.
- (4) BAVER, L. D. 1926 The use of the quinhydrone electrode for measuring the hydrogen-ion concentration of soils. *Soil Sci.* 21: 167-179.
- (5) BEAR, F. E., AND SALTER, R. M. 1916 The residual effects of fertilizers. W. Va. Agr. Exp. Sta. Bul. 160.
- (6) BLAIR, A. W., AND PRINCE, A. L. 1924 Some changes brought about in cylinder soils by long-continued crop and fertilizer treatment. *Soil Sci.* 18: 31-52.
- (7) BRIJOUX, CH. 1925 Acid soils of the Pays de Caux. *Ann. Sci. Agron. Franç. et Étrang.* 39: 129.
- (8) BROWN, P. E., AND JOHNSON, H. W. 1915 The effect of grinding a soil on its reaction. *Jour. Amer. Soc. Agron.* 7: 216-220.

- (9) BURD, J. S., AND MARTIN, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 151-167.
- (10) BURGESS, P. S. 1922 The hydrogen-ion concentration of soils as affected by drying. *Science* (n.s.) 55: 647-648.
- (11) BURGESS, P. S. 1924 The effect on present soil reaction of long-continued applications of equivalent amounts of high calcium and high magnesium limes. *Soil Sci.* 18: 169-172.
- (12) CONNER, S. D. 1915 Acid soils and the effect of acid phosphate and other fertilizers on them. *Jour. Indus. Engin. Chem.* 8: 35.
- (13) COOK, R. C. 1916 Effect of grinding on the lime requirement of soils. *Soil Sci.* 1: 95-98.
- (14) CROWTHER, E. M. 1925 The soil reaction of continuously manured plots at Rothamsted and Woburn. *Jour. Agr. Sci. (England)* 15: 222.
- (15) ERDMAN, L. W. 1921 The effect of gypsum on the soil reaction. *Soil Sci.* 12: 433-448.
- (16) GARDNER, F. D., AND BROWN, B. E. 1911 The lime requirement of the general fertilizer plots as determined periodically. *Penn. State Rpt.* 1910-11: 25.
- (17) HALL, A. D. 1917 The Book of the Rothamsted Experiments, ed. 2: Chapter 15—Secondary actions of artificial manures upon the soil. London.
- (18) HOWARD, L. P. 1919 The reaction of the soil as influenced by the decomposition of green manures. *Soil Sci.* 9: 27-39.
- (19) KARRAKER, P. E. 1923 A note on soil reaction studies. *Soil Sci.* 15: 473-478.
- (20) KELLEY, A. P. 1923 Soil acidity, an ecological factor. *Soil Sci.* 16: 41-54.
- (21) KURZ, H. 1923 Hydrogen-ion concentration in relation to ecological factors. *Bot. Gaz.* 76: 1-29.
- (22) LIPMAN, J. G., PRINCE, A. L., AND BLAIR, A. W. 1920 The influence of varying amounts of sulfur in the soil on crop yields, hydrogen-ion concentration, lime requirement, and nitrate formation. *Soil Sci.* 12: 197-207.
- (23) LIPMAN, J. G., PRINCE, A. L., AND BLAIR, A. W. 1924 Field experiments on the availability of nitrogenous fertilizers, 1918-1922. *Soil Sci.* 19: 57-79.
- (24) MATHER, WM. 1922 The effects of limes containing magnesium and calcium upon the chemical composition of the soil and upon plant behavior. *Soil. Sci.* 13: 337-354.
- (25) MORSE, F. W. 1917 Effect of fertilizers on the hydrogen-ion concentration in soils. *Jour. Indus. Engin. Chem.* 10: 125.
- (26) MORSE, F. W. 1924 The loss of CaCO_3 in drainage waters as affected by different chemical fertilizers. *Soil Sci.* 17: 249-254.
- (27) PLUMMER, J. K. 1918 Studies in soil reaction as indicated by the hydrogen electrode. *Jour. Agr. Res.* 12: 19-31.
- (28) ROST, C. O., AND FIEGER, E. A. 1923 Effect of drying and storage upon the hydrogen-ion concentration of soil samples. *Soil Sci.* 16: 121-126.
- (29) SKINNER, J. J., AND BEATTIE, J. H. 1917 Influence of fertilizers and soil amendments on soil acidity. *Jour. Amer. Soc. Agron.* 9: 25-35.
- (30) STEPHENSON, R. E. 1921 The effect of organic matter on the soil reaction. *Soil Sci.* 12: 145-162.
- (31) VEITCH, F. P. 1906 The effect of fertilizers on the soil reaction. *Science* (n.s.) 23: 710-712.

BOOK REVIEWS

Anleitung zum quantitativen agrikulturchemischen Praktikum. (Methods of analysis in agricultural chemistry.) By Prof. G. WIEGNER with the collaboration of Dr. H. JENNY. Gebrüder Borntraeger, Berlin, 1926. Pp. 348, fig. 33.

The author of this book is the well known Professor of Soil Chemistry at the Agricultural Institute in Zürich, Switzerland. He presents here, concisely and clearly, all the information and directions which are essential in quantitative analysis and its application to agricultural chemistry, especially the quantitative methods of physical and chemical analysis of soils and fertilizers. The most recent methods have been treated in a most accurate and careful manner.

The book is divided into four parts: 1. Gravimetric and volumetric methods of analysis. 2. Investigation of artificial fertilizers. 3. Physical and chemical soil investigation. 4. Chemical investigation of foodstuffs. A table of atomic weights, a 4-place logarithmic table, and excellent name and subject indices are appended.

Although written as a laboratory text for students in agricultural chemistry, the book will prove a valuable addition to every chemical laboratory where soils, fertilizers, feeds, or milk products are analyzed. No one interested in the subject, either from the point of view of teaching, analysis, or research can afford to overlook this book.

The book is well printed and the different methods are properly illustrated and clearly defined.

S. A. WAKSMAN.

The composition and distribution of the protozoan fauna of the soil. By H. SANDON. Oliver and Boyd, Edinburgh and London, 1927. Pp. xiii + 227, pl. 6, charts 3.

Although the existence of protozoa in the soil has been known since the time of Ehrenberg and Greef, and the great abundance of these organisms in the soil, both in numbers and in species, has been pointed out by Wolff and others, it was only in 1909, with the work of Russell and Hutchinson on partial sterilization of soil, that a systematic study of the distribution of protozoa in the soil was begun. These investigators advanced the theory that the favorable effect of partial sterilization of soil upon the numbers and activities of bacteria and upon plant growth is due to the elimination of protozoa, which otherwise consume the bacterial population of the soil as food. The subsequent studies on the protozoa of the soil were greatly influenced by this theory, many investigators being concerned largely with obtaining information which would

support or refute it. Other investigators, however, were more concerned with gaining further information on the physiology of protozoa, their distribution in various soil types, and their activities in the soil as influenced by environmental conditions.

The author of this monograph, a member of the Rothamsted Station, England, succeeded in carrying out by far the most extensive study of the distribution of protozoa in different parts of the world, under various environmental conditions. As a result of his own investigations and also after a thorough study of the literature, the author recognized that "the time had come when the chapter of soil protozoology could be brought to a close by bringing together all the available records, reconciling wherever possible their mutual inconsistencies and contradictions, thus presenting for the use of the soil microbiologist and the general naturalist a clear and tolerably complete picture of this group of organisms and of their distribution in the soil." The object of the work reported in the monograph was to "pave the way for more detailed inquiries . . . by giving a general review of protozoa occurring in the soil, and of the factors influencing their distribution."

The author dissociates completely the study of protozoa, a very extensive group of soil organisms which no doubt play an important rôle in the numerous soil processes, from the subject of partial sterilization of soil.

The major part of the monograph is devoted to a study of the distribution of protozoa in 148 soils collected from different parts of the world, including the polar regions and tropical regions, as well as an analysis of the factors influencing this distribution. A description of all the species so far authentically recorded from soils, and original observations concerning some new species then follow. Artificial keys are added to assist in the identification, except in the case of naked Rhizopoda, which do not lend themselves to this treatment.

The book is well printed and the six plates, some of which are original drawings of the various soil protozoa, are executed in a most excellent manner.

S. A. WAKSMAN.

CARBON DIOXIDE EVOLUTION OF SOIL AND CROP GROWTH

HENRIK LUNDEGÅRDH

Ecological Station of Hallands Väderö, Sweden and Central Experiment Station, Stockholm

Received for publication April 5, 1926

INTRODUCTION

Carbon dioxide evolution from the soil, and its relation to microbiological activity and to climatic factors have been studied by a large number of scientists (17, 34, 32, 33, 25, 6, 29), who have found a relation between the carbon dioxide evolution on one hand and the number of bacteria and the intensity of nitrification on the other hand. The influence of mineral salts, humidity, manure, and temperature on the rate of carbon dioxide evolution has been studied by Wollny (36), Petersen (25), König and Hasenbäumer (10), Van Suchtelen (33), Lundegårdh (16, 17), Waksman and Starkey (34), and others.

Most of the investigators cited above have worked with soil samples in the laboratory. After treatment with water, salts or manure a portion of 1 kgm. or less of soil was placed in a vessel and a stream of CO_2 -free air was passed through the soil. The evolved carbon dioxide was determined after absorption in alkali (32).

Potter and Snyder (27) and Neller (23) first passed the CO_2 -free air continuously over the surface of an enclosed soil sample. Lundegårdh (16) put 100 gm. soil into a 1000-cc. Erlenmeyer flask and measured volumetrically the accumulation of carbon dioxide in the flask air after 24 hours. For analysis, a 10-cc. sample of air was sucked into an apparatus of simplified Haldane type (fig. 1). This method permits work on a large scale because the flasks can be put easily into thermostats without any special arrangement for streaming air. In general, methods where the carbon dioxide is allowed to escape from the soil by means of diffusion are to be preferred to methods where an air stream passing through the soil is used. In nature, free diffusion is the chief factor in the liberation of the carbon dioxide that is accumulated in the pores of the soil (28; 17, p. 156).

THE SOIL RESPIRATION

For studies on the relation between carbon dioxide evolution and climatic factors and crop production a method is needed for estimating the "soil respiration," or the amount of CO_2 that is given off in one hour from one square meter of the soil in situ. The author used the following method (16; 17, p. 146):

The "respiration bell," consisting of a conic zinc vessel with a cylindric edge and a tube sr at the top (fig. 2), has an open width of 750 sq. cm. The edge e is 6 cm. high and the conic part 10 cm. high. When the edge is driven into the soil, the volume of air enclosed over u in the bell is about 2300 cc. Before the beginning of the experiment the surface of the soil must be smoothed in order to avoid errors in the air volume. The inside of the bell is coated with paraffin to avoid absorption of CO_2 .

After 10 to 20 minutes a small sample of the bell-air is taken into an apparatus for volumetric analysis of carbon dioxide. The author has constructed a new apparatus that permits

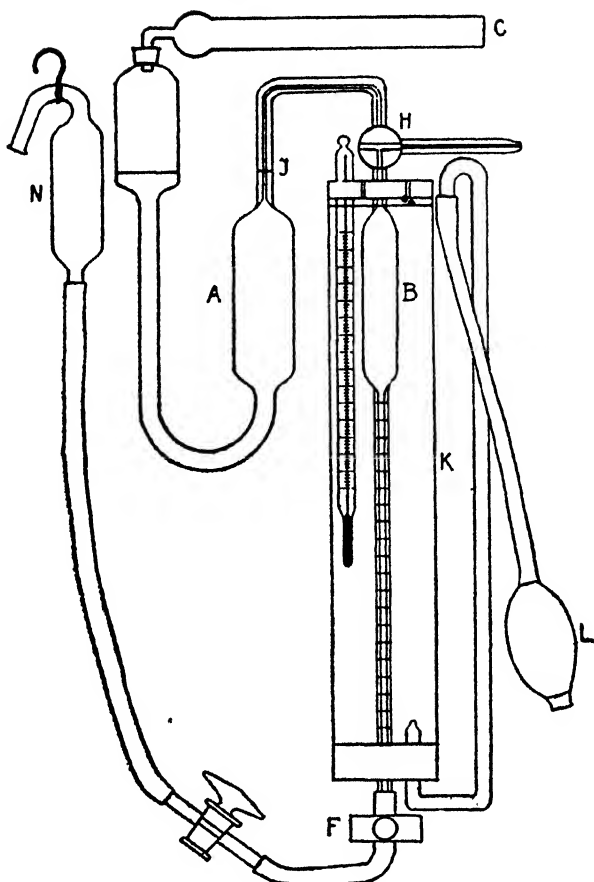


FIG. 1. SIMPLE APPARATUS FOR ANALYZING THE CO_2 CONCENTRATION OF THE AIR

B, gas burette; *A*, absorption vessel (with 20 per cent KOH solution); *I*, index; *N*, mercury vessel; *F*, screw for adjusting; *L*, rubber ball for blowing air through the water mantle *K*.

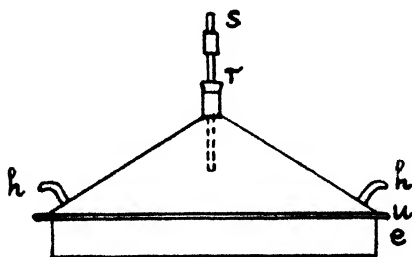


FIG. 2. APPARATUS FOR ESTIMATING THE SOIL RESPIRATION ("RESPIRATION BELL")

h, handles; *e*, cylindric edge; *u*, ring up to which *e* is driven in the soil; *sr*, tube for drawing the air sample.

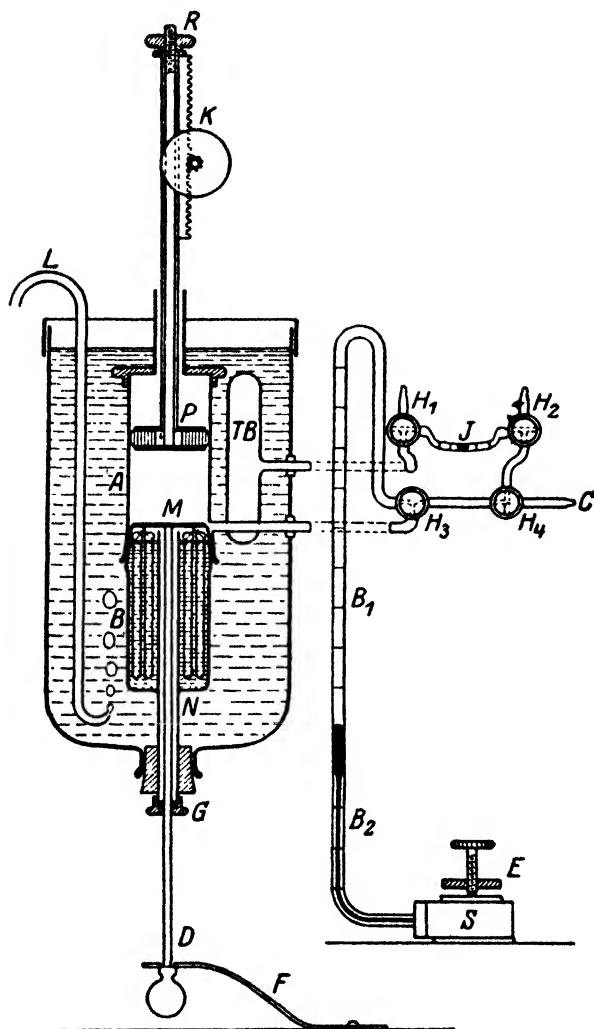


FIG 3. SCHEME OF NEW APPARATUS FOR ACCURATE ANALYSIS OF THE CO_2 CONCENTRATION IN THE AIR

A, glass cylinder; *P*, air-tight piston for filling *A* with the air sample; *B*, absorption vessel with 12 per cent KOH + 3 per cent Ba(OH)_2 , air-tight connected with *A*; *M*, metal lid for *B*, attached to the stick *MD*, and provided with glass rods dipping into the absorption solution in *B*; *N*, glass tube in which *MD* is moving; *G*, air-tight packing for *MD*; *TB*, thermobarometer for compensation of variations in air pressure and temperature; *J*, index of petroleum; *B₁ B₂*, burette; *S*, rubber vessel filled with mercury; *E*, screw for adjusting the mercury stand in *B₁ B₂*. For making the determinations see (18, p. 203).

analyses in the field with an error in single observations of only 0.003 per cent CO_2 (18, p. 203, Ab. 2). This apparatus is portable and can be placed on portable stand (fig. 3 and plate 1, fig. 1). A higher CO_2 concentration than 1 per cent in the soil atmosphere may become dan-

gerous to crop growth not only because the growth rate is retarded, but because certain parasitic fungi, as *Fusarium culmorum*, are stimulated, causing increased infection (15).

The calculation of the soil respiration from the estimated value of the carbon dioxide concentration in the bell is carried out by means of the formula

$$\frac{(a - b) \times 1.858 \times 2300 \times \frac{60}{t}}{750} = x \text{ (in gm. to the hour for each square meter)}$$

where a is the CO_2 concentration in the bell-air at the beginning, b at the end, calculated in per cent, t the time in minutes, 1.858 the weight of 1 liter CO_2 at 15°C . and 760 mm. pressure, 2300 the volume of the bell, and 750 the soil surface under the bell. Corrections for air pressure and temperature are applied as usual.

The formula is valid as long as $a - b$ is proportional to the time t . Special tests have shown that this is approximately true for 20 minutes, after which the accumulation of carbon dioxide in the bell-air causes a diminution of the rate of diffusion from the soil (16, p. 150).

TABLE 1
Soil respiration on various soils

UNTREATED SOILS	DATE	SOIL RESPIRATION PER SQUARE METER PER HOUR
		gm
Sandy soils (low humus content)	August 26, 1922	0 206
Sandy soil (4 per cent humus)	August 26, 1922	0.399
Sandy loam	February 9, 1921	0 317
Loam	November 17, 1922	0 397
Loam (10 to 15 per cent humus)	June 19 to September 13, 1923	0.411
Clay soil	June 23 to October 10, 1925	0.125
Alder forest	August 13, 1922 and October 10, 1921	1.170 to 2.340
Beech forest	August 31, 1920	2.20 to 1.54
	October 10, 1921	0.33 to 0.83
Alder swamp	October 10, 1921	0.28
Meadow with <i>Nardus</i> and <i>Carex</i>	October 10, 1921	0.33

Averages of several determinations.

In the years 1921 to 1925 the author made a great number of determinations of the soil respiration. On agricultural soils in South Sweden the respiration varied between 0.125 and 0.411 gm. to the square meter in an hour. Light sandy soils with low humus content showed a minimum respiration. The highest values were obtained on loam with 10 to 15 per cent humus (table 1). A heavy clay soil from fertile Skåne county showed a very low respiration.

Forest soils evolve in general much more carbon dioxide than agricultural soils or grass land. The difference is probably due to the greater porosity and more uniform humidity of the forest soils. The activity is always highest in the upper layer of the soil, but in the cultivated fields the periodical desiccation and the high light intensity must act very unfavorably on the micro-biological processes in the soil surface.

THE CARBON DIOXIDE CONCENTRATION IN THE SOIL AIR

The soil respiration is the total of all soil processes in which carbon dioxide is produced. Pure chemical oxidation will probably always occur (8, 10) but as a rule only to a small extent. The microflora (bacteria, actinomyces, fungi) produce the chief part of the carbon dioxide, but the plant roots in many cases also are giving off considerable quantities of this gas.

The respiration from a bare soil and that from the same soil covered with oats were compared. The former showed a respiration of 0.268 gm. and the latter a respiration of 0.399 gm., a difference of 0.131 gm.; the root respiration thus forms about 30 per cent of the total soil respiration. Further experiments indicated, however, that a great deal of what we call root respiration is due to bacteria that inhabit the root surface and probably are fed with the organic substances that are delivered from the roots (7, 35, 20).

Oats and wheat were cultivated in sterilized soil and in non-sterilized soil. The roots from the non-sterilized soil evolved about 45 per cent more carbon dioxide per unit dry substance than the roots from the sterilized soil. A kind of "Rhizosphere" (9) probably must be assumed and the total root respiration is the sum of the respiration of the living root cells and of the microorganisms that live in contact with the root surface or in the vicinity of the roots.

The carbon dioxide concentration in the soil air (the pore space) is a function: first, of the absolute CO_2 production of the soil particles and the roots (A), and secondly, of the diffusion velocity. An expression for the latter is: the diffusion coefficient K = the volume of gas (in cc.) that in 1 second passes through a cylinder of 1 sq. cm. base and 1 cm. height, when the difference in partial pressure is 1 atmosphere. In physics, K is used as a property of the diffusing gas. K here will be used for characterising the medium and will be defined as "the diffusion value of the soil" when the diffusing gas is carbon dioxide.

The simplest relation between the absolute CO_2 production (A), the diffusion value (K), and the CO_2 concentration in the soil air (C) is

$$C = k \cdot \frac{A}{K}$$

C alone can never be used as an adequate expression for the soil activity.

For measuring C Pettenkofer (26), Fodor (5), Lau (11), and Russel and Appleyard (29), have used rather complicated experimental arrangements that cannot be used far from the laboratory. Romell (28) introduces the microgas-analysis apparatus of Krogh. For drawing the gas samples he uses a thin brass tube of special construction that is put into the soil; the sample is collected in small glass tubes. The Krogh apparatus is good in cases where the air content in the soil is very small. For ordinary soils the gas-analysis-apparatus mentioned above (fig. 1 or 3) gives far more accurate values. The sample is drawn directly into the analyzing apparatus. The construction of

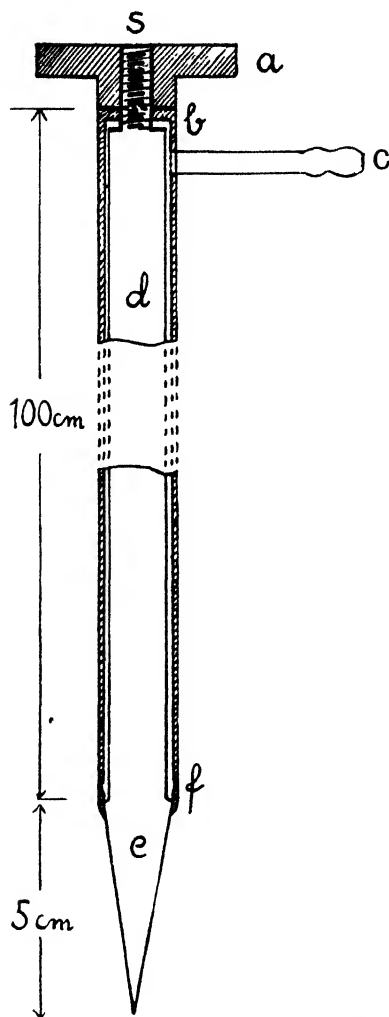


FIG. 4. TOOL FOR DRAWING AIR-SAMPLES FROM THE SOIL

A brass tube *bf*, about 1 cm. in diameter, is provided at the upper end with a small tube *c*, which is connected by a rubber tube with the analyzing apparatus. The steel rod *sde* with its conic end *e* fills up *bf*, but the air can circulate between rod and tube wall; *e* is moving tight, but 2 mm. above the edge of the conic part (at *f*) the tube wall is hollowed out. When therefore *sde* is drawn back a few mm. by aid of the screw *s* and the nut *a*, the air can pass through. Before drawing the sample, *e* must be lowered below *f*. The stick is then driven into the soil to the desired depth (usually 15 cm.). Then *e* is elevated until the upper end of *d* hits the lid of the brass tube. This closure is then air-tight while the air can pass by *f*.

the brass tube used for drawing the samples is shown in figure 4. The analysis apparatus is connected with the mouth-piece *c* by a thin rubber tube, as shown in plate 1, figure 1.

In connection with the field experiments at the station many determinations of the CO_2 concentration in the soil atmosphere have been made (17, p. 167 seq.). Only a brief review of the results can be given here.

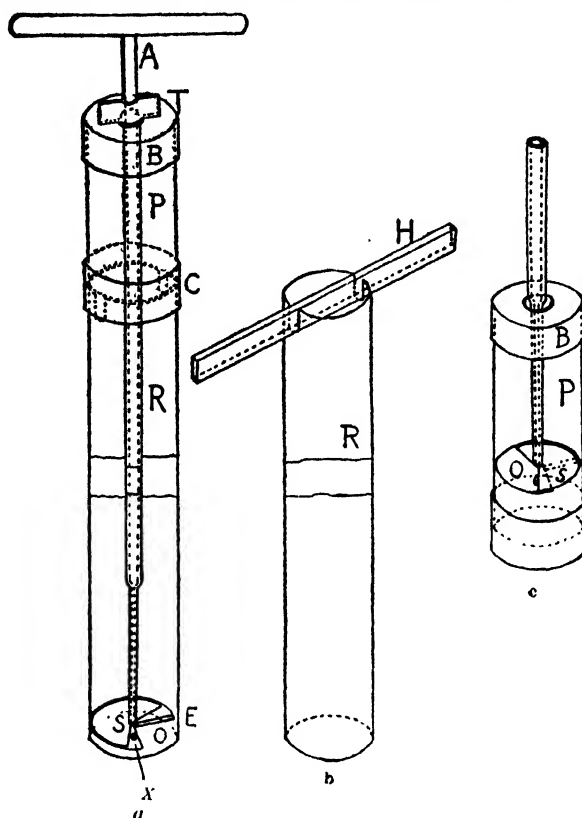


FIG. 5. BORER FOR DRAWING SOIL SAMPLES OF A DEFINITE VOLUME FROM DIFFERENT DEPTHS

It consists of two parts *R* and *P*, the former is the borer proper: a steel tube of about 1½ inches diameter and 3 feet in length. *R* has at the upper end two scores, in which the iron handle *H* is placed. The lower end is ground to an edge. When *R* has been bored into the soil, the shorter tube *P* is put on it and the boring arrangement *AS* is applied. This consists of a steel plate *E* with a sector carving at *O*, attached to a steel rod *A*. When the handle *A* is turned around, *E* will bore itself into the soil, because one side of the carving is bent down as an edge. On the steel plate another plate sector *S* is sliding. *AS* is attached to a brass tube *ST* that surrounds the rod *A*. When *E* has been bored down to the edge of *R* the small handle *T* is turned so that *S* covers *O* (see *c*). When the whole boring arrangement then is drawn back, the soil sample will slip with it. *P* is then detached from *R* and the sample is put into a box or flask.

As already stated by Lau, Russel and Appleyard, Romell, and others, the CO_2 concentration increases with the depth below the soil surface. In 15 cm. depth, *C* averages about 0.3 per cent for lighter soils, but may arise to 1

per cent or more, especially in wet soils rich in humus. Even in the same field the variation from plot to plot may be very prominent, especially in soils that are not well cultivated and contain lumps of decomposing organic matter. This is another reason why *C* ought not to be used as an indicator of the soil activity.

The *C* values are important for estimating the growth conditions of the plant roots. It is a well-known fact that higher CO_2 concentrations have an injurious effect on roots (28, 15). The resistance of different plants varies greatly. General statements of this poisonous concentration are, therefore, of no great value; the available facts seem, however, to indicate $C = 1$ per cent as the lower limit for alteration (23; 17, p. 164).

By determining the CO_2 concentration at 15 cm. depth, one can therefore detect an unhealthy condition of the soil that can be removed by cultivation, drainage, or other measures for increasing the aeration of the soil.

TABLE 2
CO₂ production in the profile of a sandy soil

DEPTH	CO ₂ PRODUCED TO THE SQUARE METER IN 1 HOUR	K
<i>cm.</i>	<i>gm.</i>	
0-10	0.271	0.0644
10-20	0.084	0.0237
20-30	0.016	0.0128
30-40	0.009	
S:a	0.380	
Direct determination of the total soil respiration	0.345	

CO₂ PRODUCTION AT DIFFERENT DEPTHS

For many purposes it is important to know the vertical distribution of the processes bringing about the evolution of CO_2 . With a borer of special construction (fig. 5) definite volumes of soil are drawn from different depths. The samples are taken to the laboratory in closed flasks and the free evolution of carbon dioxide from them is measured in the manner previously described (p. 417). After reducing the activity to unit soil volume and time the activity of the whole soil profile is reconstructed, as shown in table 2.

This experiment which has been repeated several times, makes clear: first, that almost all the CO_2 produced in the soil is given off from the soil surface; and secondly, that the moving force of the soil respiration is the diffusion.

In the case cited in table 2 the soil activity is concentrated in the surface layer and decreases very rapidly with the depth. In other cases the upper 30 cm. of the crust respire more uniformly. When the soil is manured the layer where the rotting dung lumps are lying reveals the highest activity.

DIFFUSION VALUE, AN INDICATION OF SOIL AERATION

The estimation of the total soil respiration, the absolute activity of the profile, and the CO_2 concentration in different depths enable us to calculate the diffusion value K of a stratum of the soil.

We assume that the soil is composed of several strata, S_1, S_2, S_3 , etc. (fig. 6). The activity of these strata is a_1, a_2, a_3 (calculated in cubic centimeter per hour and per square centimeter base). As the carbon dioxide diffuses upward, the CO_2 quantities $a_1 + a_2 + a_3, a_2 + a_3$, and a_3 cubic centimeters are moving through S_1, S_2 , and S_3 . Finally the CO_2 concentrations b , at p_1, p_2 and p_3 have been determined (fig. 6).

If the thickness of the stratum S_2 is y_2 cm. the following equation results:

$$K = \frac{(a_2 + a_3) \cdot y_2 \cdot 100}{3600 \cdot (b_2 - b_1)}$$

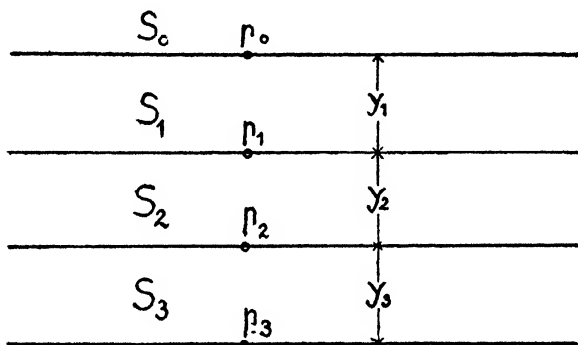


FIG. 6. SOIL STRATA

In a similar manner K is calculated for the other strata. The equation, of course, gives approximate values of K , but the error diminishes with y . In practice, determinations with smaller values of y than 5 to 10 cm. are difficult to carry out. In table 2 the K values of three strata are given.

The estimation of the diffusion values of different strata of course gives a much more adequate idea of the aeration of the soil than a determination of the average K value. Especially with forest soils it seems necessary to take into consideration the stratification, which in this case may be very prominent. Agricultural soils also may be less aerated in lower strata, as shown in table 2.

In general the structure of cultivated soils is more uniform; for practical purposes, therefore, a simplification of the formula seems justifiable. Only C at 15 cm. depth and the total soil respiration A are thus measured. After calculation of the latter in cubic centimeters to the hour (Aa) the formula will appear in this way:

$$K = \frac{Aa \cdot 15 \cdot 100}{3600 \cdot (C - 0.03)}$$

where K equals the approximate diffusion value of the upper 15 cm. of the soil. K increases with increasing A and decreases with increasing C . Assuming that the average $A = 0.4$ gm. and $C = 1.0$ per cent, the value of $K = 0.009$, which is the minimum for the normal aeration (cf. p. 424). In good soils K is always considerably higher (table 3). When A is higher than 0.4 gm., as in most forest soils, the minimum for the normal aeration is correspondingly raised.

The method for expressing and measuring the aeration of the soil is based upon the experimentally proved fact that diffusion is the moving force of the

TABLE 3
Diffusion value K

	C	A	K
	<i>per cent</i>	<i>gm.</i>	<i>gm.</i>
Loam, 1922.....	0.18	0.397	0.060
Sandy loam, 1921.....	0.30	0.399	0.033
Loam rich in humus, 1923.....	0.68	0.411	0.014
Alder forest	1.02-1.80	2.34	0.051-0.029

TABLE 4
Fluctuations of soil activity during the vegetation period

DATE	AVERAGE TEMPERATURE*	RAIN FALL	CO ₂ CONCENTRATION 4 M. ABOVE THE SOIL SURFACE†	CO ₂ CONCENTRATION 15 CM. BELOW THE SOIL SURFACE‡	DIFFUSION VALUE§	SOIL RESPIRATION PER SQUARE METER PER HOUR
1923	°C.	mm.	mgm. per l	volume per cent		gm.
June 19	12.6	0.0	0.543	0.456
June 29	12.0	4.1	0.567	0.39	0.031	0.443
July 10	20.9	5.9	0.544	0.46	0.0199	0.378
July 24	14.6	26.8	0.522	0.84	0.010	0.370
August 10	17.5	55.9	0.568	0.438
August 21	12.5	18.2	0.596	0.59	0.018	0.435
September 4	11.0	67.8	0.625	0.412
September 13	10.4	26.1	1.26	0.010	0.540

* According to thermographical record.

† Average of 71 daily analyses at the station near the seashore.

‡ Each value averages from 9 to 16 observations.

§ According to formula, page 425.

gas exchange between the soil and the atmosphere. Earlier efforts to measure the soil "permeability" (21, p. 171 seq.) have no great practical value since the gas is determined *under pressure*.

The aeration of the soil is the upward diffusion of carbon dioxide and the downward diffusion of oxygen. As a rule the amount of oxygen absorbed by the soil is equal to the amount of carbon dioxide given off. Therefore the diffusion value of carbon dioxide is a very good measure for the total aeration. Exceptions to the rule occur in very badly aerated soils (12, 28); here the oxygen absorption is usually greater than the carbon dioxide liberation.

After the soil is treated with lime, the evolution of carbon dioxide is diminished because of chemical combination. Normally only very little carbon dioxide is retained in the soil or escapes with the ground water.

The method is rather simple: the determination of the soil respiration (fig. 2) takes about 20 minutes; while the carbon dioxide is accumulating in the respiration bell, the air sample from 15 cm. depth can be drawn. *A* and *C* are important not only for calculating *K*, but also in connection with soil fertility and pathology. The methods described in this paper are useful in studying the condition of the soil, especially in the case of a number of nonparasitic

TABLE 5
Fluctuations of soil activity during the vegetation period

DATE	AVERAGE TEMPERATURE	TEMPERATURE 5 CM. BELOW THE SOIL SURFACE	RAIN-FALL	CO ₂ CONCENTRATION 4.5 M ABOVE THE SOIL SURFACE	CO ₂ CONCENTRATION 0.25 M ABOVE THE SOIL SURFACE	SOIL RESPIRATION PER SQUARE METER PER HOUR
1925	°C	°C.	mm.	mgm. per l	mgm. per l	gm.
June 23-26	16 15*	34 0	0.571†	0.5948‡	0.160§
June 29-July 1	19 1	0 0	0.624	0.6480	0.180
July 7-9	16 98	13 0	0.656	0.6474	0.188
July 13-15	20.4	0 0	0.671	0.6580	0.256
July 23-24	23 3	19 8-20 0	0 0	0.664	0.5962	0.228
July 28-29	14 4	19 0-18 6	24 0	0.611	0.6025	0.200
August 4-5	18 1	16 7-16 2	2 0	0.581	0.5764	0.246
August 11-13	18 3	19 5-17 1	7.0	0.776	0.7640	0.192
August 19-20	17.3	15 7-15 0	9 0	0.717	0.7441	0.318
August 26-27	16.1	16 0-15 4	4 0	0.559	0.6088	0.148
September 1-2	11 9	14 3-13 5	39 0	0.585	0.6200	0.246
September 10-12	10 0	11 2-10 7	28 0	0.570	0.5795	0.188
September 15-17	13.0	11 2-12.2	2.0	0.567	0.5677	0.204
September 22-24	11 6	12 1-12 5	12.0	0.575	0.5844	0.160
September 29-October 1	10.8	11 6-11.3	10 0	0.597	0.6152	0.290

* Air temperature according to thermographical record. Average for 1 week.

† Average for 1 week, from the field laboratory.

‡ Average for 1 week, from 9 parallel plots of the experimental field (thus 50 to 60 single analyses each).

§ Average for 2 days from 5 parallel plots. Each single observation duplicated (thus 20 single analyses each).

diseases and the conditions for infection by *Fusarium* and other organisms in the living soil (15).

THE SOIL RESPIRATION UNDER THE INFLUENCE OF CLIMATIC CONDITIONS AND FERTILIZERS

Seasonal fluctuations

Several investigators have stated that the urea-decomposing and the nitrogen-fixing powers of the soil (13, 22) and the number of bacteria (3) reveal seasonal fluctuations.

The curves show maxima in spring and fall and a depression in summer. Russel and Appleyard (29) obtained a similar curve for the carbon dioxide concentration in the soil atmosphere. Similar periods have been recorded for water organisms (4, 24).

Hitherto investigations concerning seasonal fluctuations of the total soil

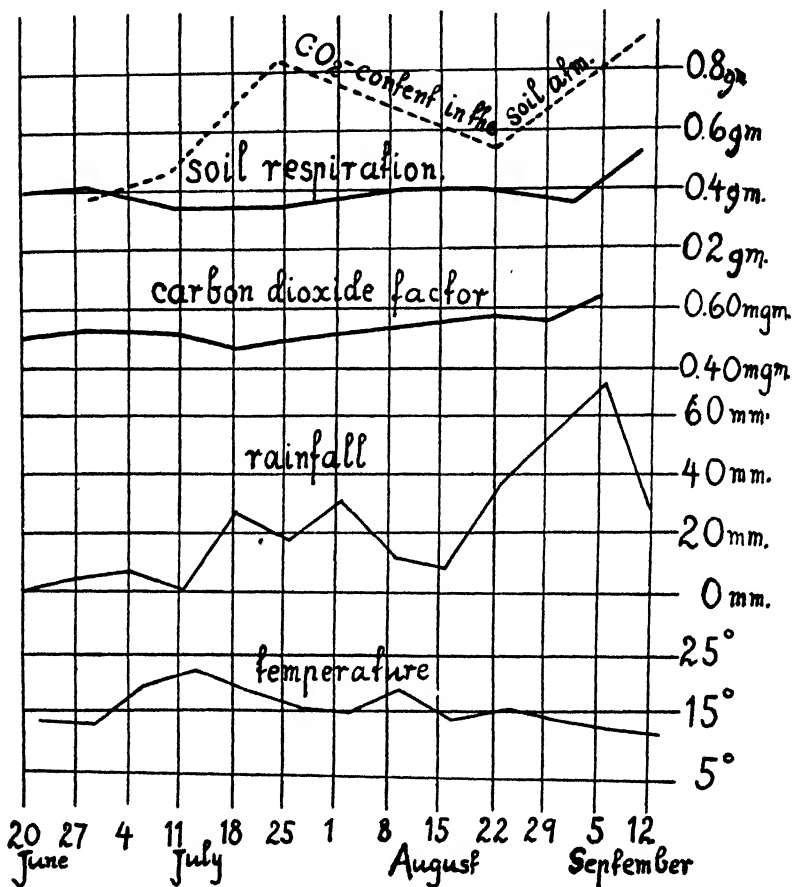


FIG. 7. SEASONAL FLUCTUATIONS OF THE SOIL RESPIRATION, CARBON DIOXIDE CONCENTRATION AMONG THE LEAVES, CARBON DIOXIDE CONTENT IN THE SOIL AIR, AND CLIMATIC FACTORS—OATS, 1923

activity have failed. The author's observations include only the vegetation period (tables 4 and 5, and figs. 7 and 8).

The most striking thing about the two curves in figures 7 and 8 is the lack of, or the incomplete, correlation between CO₂ factor and the temperature. The temperature of the upper 5 to 10 cm. where the maximal activity is located follows fairly closely the temperature of the air recorded about 15 cm. above

the soil surface (table 5), but the amplitude of the fluctuations is smaller. Nevertheless the rise in the temperature curve in July, 1923 (fig. 7) does not induce an increase in the soil respiration. In 1925 a certain correlation perhaps exists, but the remarkable increase of the soil respiration in the middle of August is apparently not due to the temperature.

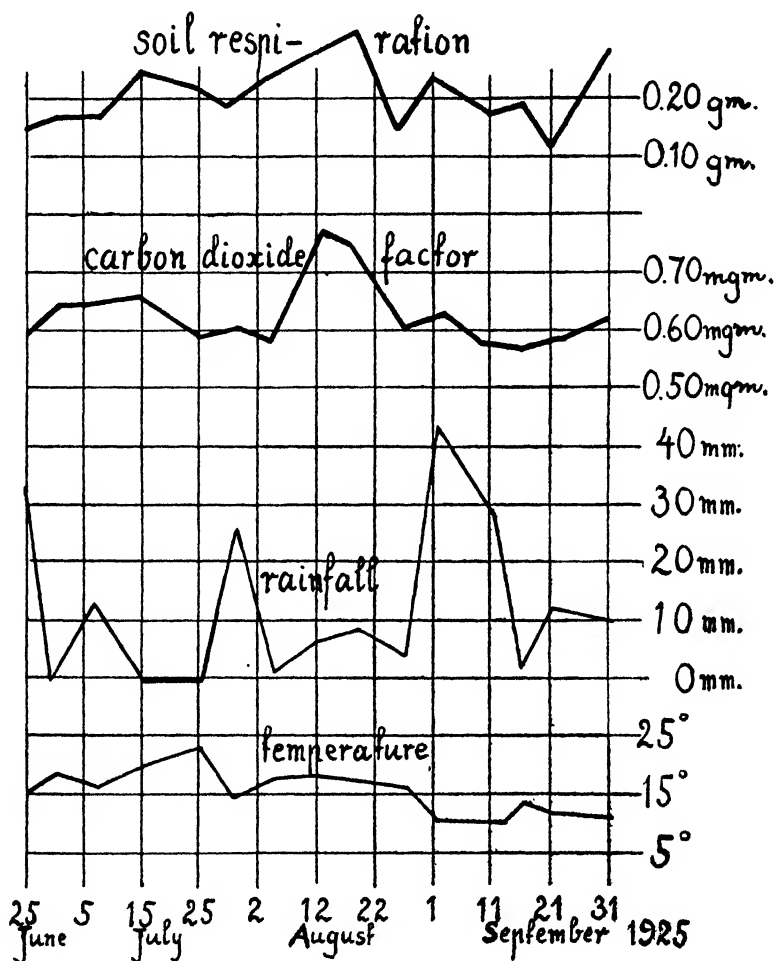


FIG. 8. SEASONAL FLUCTUATIONS OF SOIL RESPIRATION, CARBON DIOXIDE FACTOR, AND CLIMATIC CONDITIONS—SUGAR BEETS, 1925

Even rainfall is not correlated to any great extent with the total soil activity. That, on the other hand, the concentration of the carbon dioxide in the soil atmosphere and therefore also the diffusion value, rise with the soil water (fig. 7) is not surprising, because the air content in the soil decreases with increasing water content.

Both curves for the soil respiration, for 1923 and 1925, show a slight maximum in August, and both have a steep rise in September, in accord with earlier statements on bacterial activity. The cause of this periodicity is as yet unrevealed. The competition between the different types of the soil population and also the relations between microorganisms and the growing crop probably cause fluctuations in the activity, but the power of the single components in this very complicated process cannot be traced exactly (30, p. 279; 17, p. 179).

Action of fertilizers and manure

Organic matter is the source of carbon dioxide evolution, hence the humus content in soil is always a factor in the process of soil respiration. Very frequently the humus content is a factor in the maximum, hence its relative influence is small. If a humus content of 3 per cent is assumed, one hectare of soil will contain about 200,000 kgm. organic matter, which is equivalent to about 300,000 kgm. carbon dioxide. A cultivated field of this size evolves about 10,000 kgm. of carbon dioxide a year. It is easy to understand why a rise in the humus content above about 3 per cent will not change the soil respiration to any extent. As previously stated, the low respiration of poor sandy or clay soils with a low humus content is partly due to the fact that the organic matter here begins to act as a limiting factor. The author has found, however, that even in such poor soils the soil respiration will increase when mineral salts are added. The humus content itself will therefore probably very seldom act as an absolute limiting factor. When we find that the application of farmyard manure frequently changes the rate of carbon dioxide evolution, we must, as a rule, look for other causes than the increase in absolute humus content.¹

In the author's field experiments during 1921 to 1925, 10,000 to 65,000 kgm. of farmyard manure was given per hectare, and the soil respiration in the succeeding season was studied in comparison with control plots. The results from 1921 to 1923 are fully treated in previous papers (16, 17), briefly reviewed herewith.

When small and medium quantities of farmyard manure were added in the spring to sands or loams not too poor in humus, the effect upon the soil respiration was surprisingly small. The dunged plots at first evolve less carbon dioxide than the control plots, then the respiration again rises.

Large quantities of manure always increase the soil respiration (fig. 9), but the action of the manure seems to decline. The results seem to indicate the following theory: The native microorganisms of the farmyard manure persist only when large quantities are given, so that the manure is composed of coherent lumps. If the manure is uniformly spread out among the soil particles, the original environment of the manure organisms is destroyed and it will take some time for them to invade the new substrate. Part of the action of the manure is undoubtedly due to the amount of ammonia and other inorganic components (1). Moreover

¹ A rather large quantity of manure—45,000 kgm. per hectare—was added to a soil containing about 3 per cent organic matter.

the manure contains many different organic components that may act as stimulants or have a retarding effect on the soil microorganisms. Very little, however, is known about that.

The favorable effect of certain mineral salts used in the common artificial fertilizers has already been stated. The author's extensive field experiments from 1921 to 1925 prove the importance of mineral salts for increasing the soil activity (17, p. 190-197). In table 6 a selection of these experiments is summed up.

The pronounced effect of the manure in the sugar beet field in 1925 may be caused by the low humus content and the dense structure of the clay soil. The absolute activity of this soil was not equal to half of that of the sandy loam rich in humus. The estimations may have been made over a spot where the fertilizer was not well distributed.

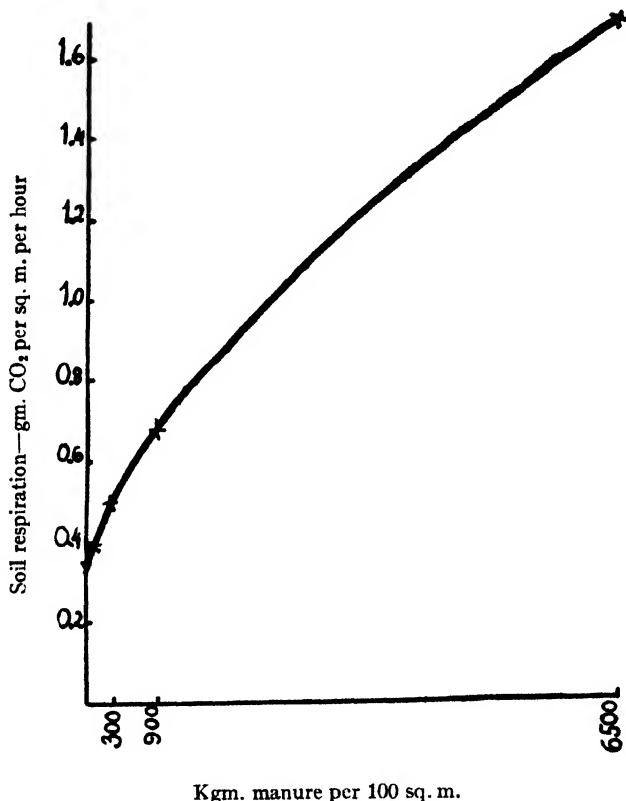


FIG. 9. THE EFFECT OF LARGE QUANTITIES OF FARMYARD MANURE ON THE SOIL RESPIRATION

SOIL RESPIRATION AND CROP GROWTH

The carbon dioxide concentration in the atmosphere as a growth factor

The importance to agriculture of the soil respiration lies in the fact that the carbon dioxide concentration in the atmosphere among the plant leaves is at a minimum (17). Rather small variations in the carbon dioxide content of the atmosphere involve corresponding variations in the rate of carbohydrate assimilation and growth.

TABLE 6
Increase in soil activities due to mineral salts

SOIL	CROP	FERTILIZER PER 100 SQ. M.	SOIL RESPIRATION PER SQUARE METER PER HOUR		TIME
			gm.	per cent	
Sandy loam, 10 per cent humus	Cabbage	<i>kgm.</i> No fertilizer	0.28*	100	1922 July 11 to August 8
		3-superphosphate 2-40 per cent potassium chloride 3-sodium nitrate	0.83	297	July 11 to August 8
		3-superphosphate 2-40 per cent potassium chloride 3-sodium nitrate 600-farmyard manure	1.0	357	July 11 to August 8
Sandy loam, 10 per cent humus	Oats	No fertilizer	0.411	100	1923 June 19 to September 13
		3-superphosphate 3-40 per cent potassium chloride 3-sodium nitrate	0.468	114	June 19 to September 13
		3-superphosphate 3-40 per cent potassium chloride 3-sodium nitrate 300-farmyard manure	0.515	125	June 19 to September 13
Clay, soil, fertile	Sugar beets	No fertilizer	0.125	100	1925 June 23 to October 1
		4-superphosphate 4-40 per cent potassium chloride 4-sodium nitrate	0.150	120	June 23 to October 1
		4-superphosphate 4-40 per cent potassium chloride 4-sodium nitrate 200-farmyard manure	0.152	121.5	June 23 to October 1
		4-superphosphate 4-40 per cent potassium chloride 4-sodium nitrate 400-farmyard manure	0.154	123	June 23 to October 1
		400-farmyard manure	0.190	152	June 23 to October 1

* These values are the averages of a great number of observations in the periods mentioned.

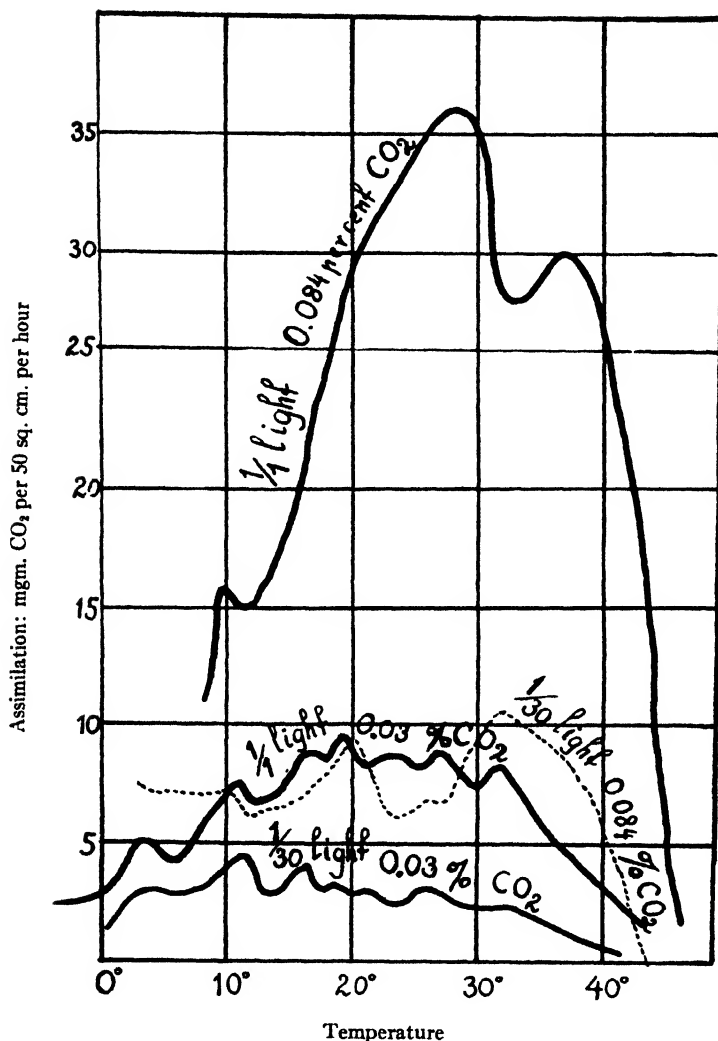


FIG. 10. THE CARBOHYDRATE ASSIMILATION OF LEAVES OF SUGAR BEETS IN RELATION TO THE FACTORS OF LIGHT, CARBON DIOXIDE, AND TEMPERATURE

The light intensity 1/1 is full daylight (sun + diffuse light), 0.03 per cent is the "normal" concentration of carbon dioxide in the atmosphere.

The influence of light, temperature, and carbon dioxide concentration upon the process of assimilation has been discussed (18). Figure 10 shows the results of a series of experiments with sugar beet leaves made in the summer of 1925 and not yet published. The curve system shows clearly the following peculiarities, stated in a previous paper (18):

1. A rise in the carbon dioxide concentration involves an increase in the assimilation not only with maximum light but also at low light intensity (one-thirtieth of full daylight).

2. The temperature optimum of the assimilation is at no fixed point, but is a function of the other factors, so that in general the optimum lies at a lower temperature when the intensity of light and the carbon dioxide factor are diminished.

3. A low light intensity (one-thirtieth of the full daylight) can be compensated by a high carbon dioxide concentration (0.084 per cent).

4. The quotient of utilization $Ak = \frac{\Delta E}{\Delta F}$ — where ΔE is the rise in assimilation intensity obtained when the carbon dioxide concentration is raised the small amount ΔF —is not constant in the whole temperature province, but is higher at 30° than at 20°, when the light is full.

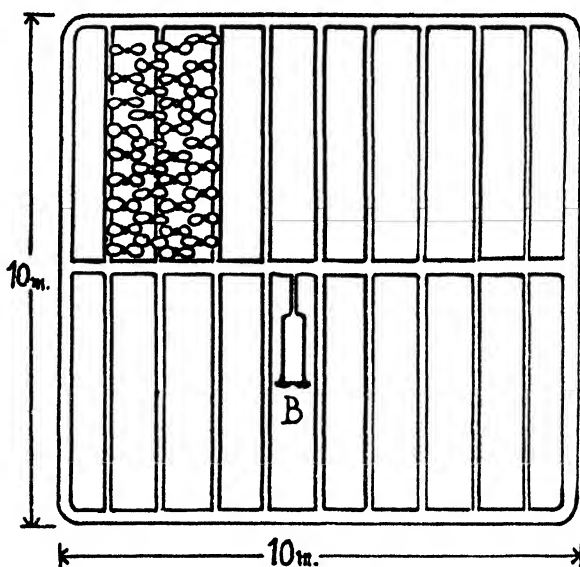


FIG. 11. SYSTEM OF PERFORATED IRON TUBES FOR GASSING OF FIELD PLOTS

B is the steel flask containing carbon dioxide

As carbohydrate assimilation is the chief nutrition process in the plant, the circumstances mentioned here will be of great importance in the understanding of plant growth. This has been proved by experiments in the field or in glass houses, where the amount of carbon dioxide in the atmosphere among the plant leaves was varied.

In 1922 and 1923, experiments with CO_2 fertilization were carried out in the field. For this purpose five 100-sq. m. plots were provided with systems of iron tubes, perforated and connected with a steel flask containing pure carbon dioxide (fig. 11). Five plots of the same size served as controls; all were cultivated with the same crop and were otherwise uniformly treated. The results given in table 7 show a clear positive effect of the gas.

These results nevertheless are not sufficient for an accurate calculation of the Ak values. More suited for this purpose are the experiments in greenhouses where the CO_2 concentration throughout the vegetation period can be held more constant than in the field.

Glasshouse experiments with cucumbers, tomatoes, beans, and sugar beets were carried out during 1921 to 1923 and in 1925. The carbon dioxide con-

TABLE 7
Results of CO_2 fertilization in the field

BEETS, 1922	FULL MINERAL FERTILIZER	FULL MINERAL FERTILIZER + CARBON DIOXIDE FROM MANURE IN ASPHALTED FELT BETWEEN THE ROWS	INCREASE IN THE PLOTS RECEIVING CO_2
Average yield of roots.....	379 4 kgm.	451 2 kgm.	per cent 19
BEETS, 1923	FULL MINERAL FERTILIZER	FULL MINERAL FERTILIZER + 40 KGM. PURE CARBON DIOXIDE FROM STEEL FLASKS	INCREASE IN THE PLOTS RECEIVING CO_2
Average yield of roots	369 6 kgm.	419 0 kgm.	per cent 16
CO_2 concentration in the air among the leaves*.....	0.520 mgm. per liter	0 600 mgm. per liter	14 5
OATS, 1923	MINERAL FERTILIZER	MINERAL FERTILIZER 20 KGM. CARBON DIOXIDE FROM JULY 28 TO AUGUST 17	INCREASE IN THE PLOTS RECEIVING CO_2
Average yield, green	46.0 kgm.	60.0 kgm.	per cent 30 5
CO_2 concentration among the leaves* ..	0 517 mgm. per liter	1.088 mgm. per liter during the gassing	

* Average from daily analyses in the field.

N. B. Attention is called to the first experiment where manure was used as a source of CO_2 . The manure was isolated from the soil by asphalted felt, placed in furrows dug between the rows of plants (fig. 12). This experiment proves that the carbon dioxide evolved from decomposing organic matter has the same effect as carbon dioxide produced from inorganic sources.

centration in the gassed house and in the control house was determined by analyses once or twice a day. The two houses were otherwise uniformly treated. The results of the experiments with cucumbers are given in table 8, and with sugar beets, in table 9.

These experiments indicate Ak values of 1.45 to 1.67, for cucumber, of 1.20 to 1.28 for sugar beets. Approximative calculations for the field experiments cited above give Ak values of about 0.60 to 0.75. The difference is probably

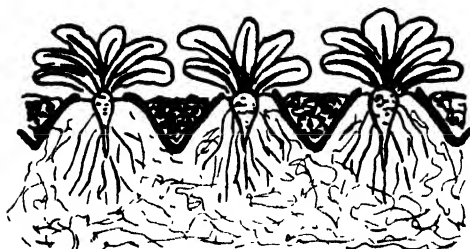


FIG. 12. ARRANGEMENT FOR GASSING OF BEETS WITH CARBON DIOXIDE EVOLVED FROM MANURE

The manure, mixed with soil, is put into furrows that are coated with asphalted felt and is consequently isolated from the soil. The carbon dioxide produced from it escapes into the atmosphere and enriches the air among the plant leaves.

due to the fact that *Ak* for the carbohydrate assimilation increases with temperatures up to 30°C. The temperature in the glasshouses was in general of the latter order, whereas the temperature in the field averaged 12 to 20°C. (see table 5).

TABLE 8
Cucumbers in glasshouses

	YIELD OF	GASSED HOUSE	CONTROL	INCREASE
				<i>per cent</i>
1922	Cucumbers	150.92 kgm.	100.49 kgm.	50
	Average CO ₂ concentration	0.044 volume per cent	0.034 volume per cent	30
1923	Cucumbers	25.879 kgm.	15.187 kgm.	74
	Average CO ₂ concentration	0.065 volume per cent	0.043 volume per cent	51

TABLE 9
*Sugar beets in glasshouses**

1925	HOUSE 1	HOUSE 2	HOUSE 3
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Average yield, absolute (<i>roots</i>)	794	1148	1211
Average yield, relative (<i>roots</i>)	100	145	152
	<i>mgm. per liter</i>	<i>mgm. per liter</i>	<i>mgm. per liter</i>
Average CO ₂ concentration, absolute	0.6806	0.7705	0.8652
Average CO ₂ concentration, relative	100	113.2	127.1
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Average yield, absolute (<i>leaves</i>)	908	1195	1428
Average yield, relative (<i>leaves</i>)	100	132	157

* The plants were cultivated in vessels each containing about 30 liters of soil, 7 plants in each house.

Soil respiration as the source of atmospheric CO₂

The total carbon dioxide evolution from the soil attains very high values. The author's investigations demonstrate values from 1.25 to 13.6 kgm. CO₂ per hectare per hour or 0.63 to 6.4 million liters in 90 days. The highest values refer of course to heavily fertilized soils, but forest soils also often attain as high values. In comparison, mention might be made that a growing field of oats consumes about 10,000 kgm. (2 million liters CO₂) in the vegetation period. The soil, therefore, frequently is producing as much carbon dioxide as the crop is using, a fact that can be generalized for the whole earth, because the living organisms after death, as a rule are completely decomposed. It is only in peats and in such deposits as charcoal that non-decomposed organic matter is stored up.

The total amount of carbon dioxide in the atmosphere is estimated at about 2100 billion kgm. (31). Growing plants take yearly about one-thirty-fifth

TABLE 10
Variations in free atmosphere during the summer period

	1920	1921	1922	1923	1924
CO ₂ content ... $\begin{cases} \text{mgm. per liter} \\ \text{per cent of volume} \end{cases}$	0 612 0 03295	0.5603 0 03031	0.5267 0 02843	0 5565 0.0300	0 6189 0.0331
Temperature °C.	19.1	17 3	16.5	15.4	16 4
Maximum variation..... per cent	75 6	90 0	108 9	77.6	60.4
Mean variation per cent	±16.1	±15 2	±14.4	±21 0

of this quantity, and about the same amount is restored by soil respiration. As the assimilation and the soil respiration do not vary according to the climatic factors, variations must be expected in the average percentage of carbon dioxide of the free atmosphere from year to year and also from season to season. Levy has shown such variations at Montsouris. The author's observations in the summer period at the Station of Hallands Väderö for the free atmosphere near the sea level gave the averages shown in table 10.

It cannot be assumed therefore that the amount of carbon dioxide in the atmosphere is constant. It varies from year to year up to 16.6 per cent (cf. 1922 and 1924) and considerably from day to day. As stated in a previous paper, the variations are related to climatic conditions, especially light and rainfall, that influence the assimilation or the soil respiration (17).

In order to measure more accurately the concentration of the carbon dioxide among the plant leaves, which of course is the real carbon dioxide factor of assimilation and growth, extensive series of analyses were made in the years 1921 to 1925. For the analyses the author used a new apparatus, consisting

of a gas-holder, in which about 2.3 liters of air stands for several hours over a free surface of diluted baryta solution (fig. 13). The baryta from which free air is excluded is then drawn off into small flasks that are transported to the laboratory and titrated. From 10 to 14 such apparatuses are left standing in the field, protected from the weather by a wooden cupboard (fig. 14). The air sample is drawn through a glass tube about 5 m. long and 3 mm. in diameter,

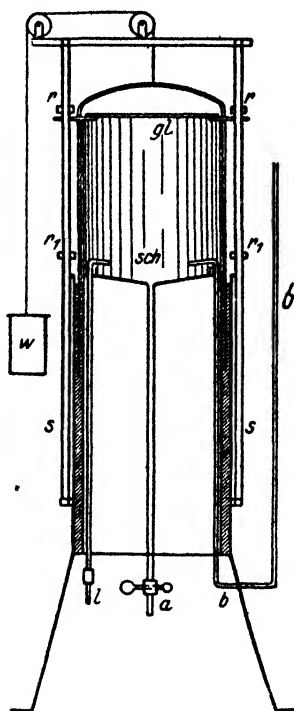


FIG. 13. APPARATUS FOR ANALYZING THE CARBON DIOXIDE CONTENT IN THE FIELD

A metal bell is suspended in glycerine. The air sample is drawn through the tube *l* by applying the weight *W*. The volume is determined by the offsets *rr* and *r₁r₁*. When the sample is taken, baryta solution is poured into the bowl *sch* through *bb*. After a few hours the baryta solution from which free air is excluded is poured out through *a* into a small flask and then titrated with 0.025 *N* HCl.

from close to the assimilating plants (fig. 14). This apparatus is working very satisfactorily, with an error of only about ± 0.005 mgm. CO₂ per liter air or ± 0.0003 per cent of volume for single observations. Thus even very small variations in the CO₂ factor can be traced. In plate 1, figures 2 and 3, is shown the field laboratory, where the chemical work is done.

The results from 1921 to 1923 are exhaustively dealt with in a previous paper (17). A few results are shown in tables 4, 5, and 10 and in figures 7 and 8.

The tables and the figures show rather clearly that the variations in the CO_2 concentration among the leaves and even in the free atmosphere above the plants are related to corresponding variations in the soil respiration. The

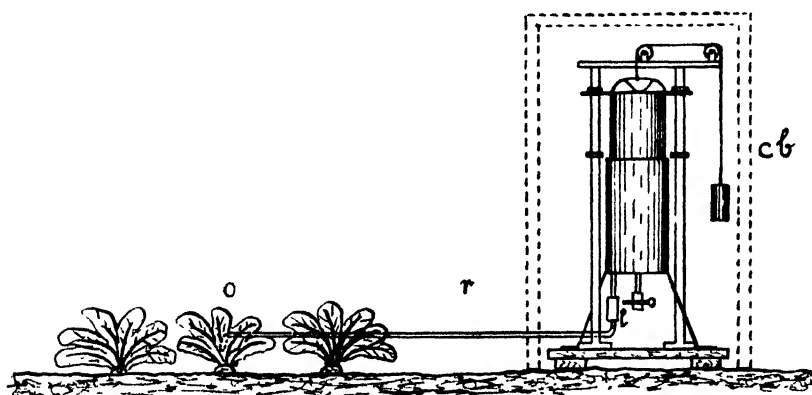


FIG. 14. ARRANGEMENT FOR SECURING THE AIR SAMPLES IN THE FIELD

cb, the apparatus (see fig. 13) protected under a cupboard; *r*, a 5 m. long, narrow glass tube, the end of which (*O*) is bent down among the leaves.

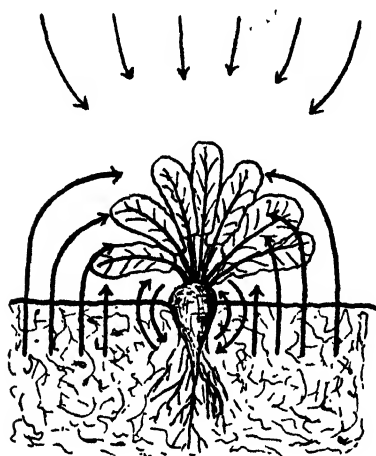


FIG. 15

FIG. 15. DIAGRAM OF THE DIFFUSION CURRENTS WHEN THE CARBON DIOXIDE FACTOR IS BELOW THE STANDARD VALUE

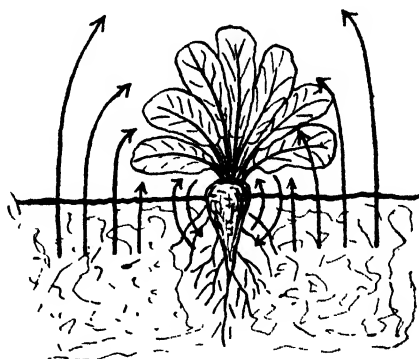


FIG. 16

FIG. 16. DIAGRAM OF THE DIFFUSION CURRENTS WHEN THE CARBON DIOXIDE FACTOR EXCEEDS THE STANDARD VALUE

correlation is very impressive, if plots of the same field receive different fertilizer treatments. When the soil respiration is stimulated by applications of mineral fertilizers (cf. p. 430), the CO_2 concentration among the leaves

in most cases will be raised too. With beets that are growing rapidly, however, the CO_2 factor may be decreased, because the rapidly growing leaves are absorbing more carbon dioxide than the soil can produce.

In studying the relations between the intensity of soil respiration and the intensity of the CO_2 factor in the level of the assimilating leaves, there is one point of special interest that throws light upon the important problem of diffusion in the atmosphere.

As long as the CO_2 factor is lower than the CO_2 concentration in the free air ("standard value," measured simultaneously 5 m. above the ground), there will be a diffusion of carbon dioxide downward, as illustrated by the arrows in figure 15. All carbon dioxide that comes from the soil will in this case be absorbed by the plants.

When the CO_2 factor exceeds the standard value, there will be a diffusion stream from the level of the leaves up to the free atmosphere (see the arrows in fig. 16); part of the carbon dioxide evolved from the soil in this case will escape between the leaves and disappear into the free atmosphere.

Table 11 shows between the soil respiration and the CO_2 factor a strikingly constant ratio until the standard value is reached by the CO_2 factor. The ratio suddenly rises in the two first series. In the third series with oats (1923) no such rise is observed. In the fourth series with sugar beets (1925) the standard value had already been attained in the plots that had received no fertilizer, but here too, the ratio rises with the increasing CO_2 factor.

From the fact stated in table 11 that a distinct relation prevails between soil respiration and the CO_2 factor in the level of the assimilating leaves, it must be concluded that the CO_2 factor and, thus, the carbohydrate assimilation of the plants are essentially regulated by the carbon dioxide evolution from the soil under the plants. The soil respiration is equal to the assimilation (cf. p. 437), and while this carbon-source for the leaves is the nearest it is the dominating one. Carbon dioxide is of course taken up from the free atmosphere too, but not to any very great extent, especially when the CO_2 factor is higher than the standard value. If the soil respiration fails to furnish a sufficient quantity of CO_2 , the supply from the atmosphere (cf. fig. 15) is furnished too slowly to prevent a CO_2 deficit among the leaves and thus a partial starving.

The plants are more or less isolated from the higher strata in the atmosphere and the circulation of the carbon in nature is most rapid in the ground stratum. This is dependent upon the fact that not diffusion but convection currents are the chief mode of carbon dioxide dissemination, and that these currents (winds) move in horizontal rather than in vertical directions. Furthermore the friction is very high among the plants. The difference between the CO_2 factor and the standard value is diminished when the weather is very windy, but is seldom eliminated. The mean difference in the vegetation period is considerable even on bleak places (17 p. 256) when a discrepancy between soil respiration and assimilation prevails.

The conclusions drawn in this paper are based upon continuous observations

at 42 points on differently treated plots in crop fields and at several points in the forest. During the vegetation period from each point were made series of daily observations of the carbon dioxide concentration, light intensity, rainfall, humidity, temperature, atmospheric pressure, wind velocity, and wind direc-

TABLE 11
Reaction between soil respiration and CO₂ factor
First series—Oats, 1921

	INCREASING FERTILIZATION				
	1	2	3	4	5
Soil respiration gm. per sq. m. per hour	0.348	0.372	0.408	0.417	0.572
CO ₂ factor (standard value = 0.026) per cent	0.0216	0.022	0.0249	0.0274	0.0284
Ratio	16:1	17:1	16:1	15:1	20:1

Second Series—Cabbage, 1921

	INCREASING FERTILIZATION			
	1	2	3	4
Soil respiration gm. per sq. m. per hour	0.224	0.273	0.284	0.590
CO ₂ factor (standard value = 0.0335) per cent	0.0305	0.0316	0.0346	0.0365
Ratio	14:1	12:1	12:1	16:1

Third series—Oats, 1923

	INCREASING FERTILIZATION		
	1	2	3
Soil respiration gm. per sq. m. per hour	0.291	0.404	0.521
CO ₂ factor (standard value = 0.0290) per cent	0.0186	0.0244	0.0358
Ratio	16:1	16:1	15:1

Fourth series—Sugar beets, 1925

	INCREASING FERTILIZATION				
	1	2	3	4	5
Soil respiration gm. per sq. m. per hour	0.200	0.270	0.264	0.290	0.324
CO ₂ factor (standard value = 0.0331) per cent	0.0334	0.0340	0.0336	0.0345	0.0348
Ratio	6:1	8:1	7.9:1	8.4:1	9.3:1

All values are means from series.

tion. Special series deal with the changes in the carbon dioxide concentration from noon to afternoon and night, and the distribution of the gas at different heights above the soil surface up to the top leaves and the free air. Only a few of the chief results have been reported here.

TABLE 12
Extract of the results concerning mean values of the CO₂ factor

		MAXIMUM	MINIMUM	MEAN AND STANDARD DEVIATION		RELATIVE VALUE
		mgm.	mgm.	\bar{e} mgm.	σ mgm.	
1921	<i>Free air</i>	0.76	0.40	0.560 ± 0.0126	± 0.090	100.0
	Potatoes, weak fertilizer	1.04	0.25	0.542 ± 0.023	± 0.161	90.0
	Cabbage, weak fertilizer	1.01	0.30	0.568 ± 0.016	± 0.109	95.3
	Cabbage, heavy fertilizer	1.34	0.42	0.680 ± 0.029	± 0.207	112.7
1922	<i>Free air</i>	0.71	0.34	0.527 ± 0.008	± 0.080	100.0
	Oats, no fertilizer	0.63	0.11	0.408 ± 0.018	± 0.126	77.4
	Oats, fertilized	0.81	0.30	0.541 ± 0.014	± 0.100	102.7
	Potatoes, no fertilizer	0.74	0.10	0.308 ± 0.019	± 0.112	58.3
	Potatoes, fertilized	0.85	0.10	0.364 ± 0.039	± 0.200	69.0
1923	<i>Free air</i>	0.71	0.47	0.556 ± 0.007	± 0.080	100.0
	Oats, no fertilizer	0.97	0.12	0.344 ± 0.040	± 0.240	61.9
	Oats, fertilized	1.17	0.35	0.662 ± 0.039	± 0.204	119.1
	Potatoes, no fertilizer	1.07	0.11	0.511 ± 0.045	± 0.270	92.9
	Potatoes, fertilized	1.67	0.60	0.661 ± 0.047	± 0.294	120.2

Dr. B. The oats (1922 and 1923) were fertilized with 300 kgm. manure to each 100 sq. m. + superphosphate, potassium chloride, and sodium nitrate, 3 kgm. to the 100 sq. m. The potatoes were fertilized with 300 kgm. manure and 2 kgm. superphosphate, 1.5 kgm. potassium chloride, and 2 kgm. sodium nitrate. The sugar beets were fertilized with 4 kgm. each of superphosphate, potassium chloride, and sodium nitrate. All plots were fertilized in the spring.

TABLE 12—*Continued*

		MAXIMUM	MINIMUM	MEAN AND STANDARD DEVIATION		RELATIVE VALUE
		mgm.	mgm.	ϵ mgm.	σ mgm.	
1925	<i>Free air</i>	0.876	0.425	0.6163 ± 0.004	± 0.041	100.0
	Sugar beets, no fertilizer	1.032	0.443	0.6215 ± 0.022	± 0.242	100.8
	Sugar beets, 400 kgm. manure	0.989	0.314	0.6304 ± 0.010	± 0.111	100.4
	Sugar beets, mineral fertilizer, + 400 kgm. manure	0.979	0.422	0.6459 ± 0.011	± 0.121	106.95

Soil respiration and fertility

It has been shown that: 1. Fertilization as a rule raises the soil respiration; 2. The CO_2 factor is largely dependent upon the soil respiration; and 3. The CO_2 factor is an important growth-factor. Thus fertilization of the soil will serve as an indirect CO_2 fertilizer (table 12).

The experiments have shown that over soils that have received no manure or fertilizer the CO_2 factor as a rule is lower than the standard value. For comparison, the latter is marked 100.0 (table 12, last column). This is the normal value of the CO_2 factor, but (to repeat) is frequently not attained over unfertilized soils where the soil respiration is not sufficient for the needs of assimilation, especially where the plants are standing close together, so that the air circulation slackens. On the same soil, therefore, oats show the lowest CO_2 factor, then potatoes; whereas beets, especially sugar beets (table 12), will permit sufficient air circulation to maintain a rather normal CO_2 factor even over unfertilized soils (17, p. 248).

Fertilization will increase the CO_2 factor more or less up to the standard value. According to figures 15, 16 and the discussion above, the CO_2 factor will rise more easily up to 100 than over 100.

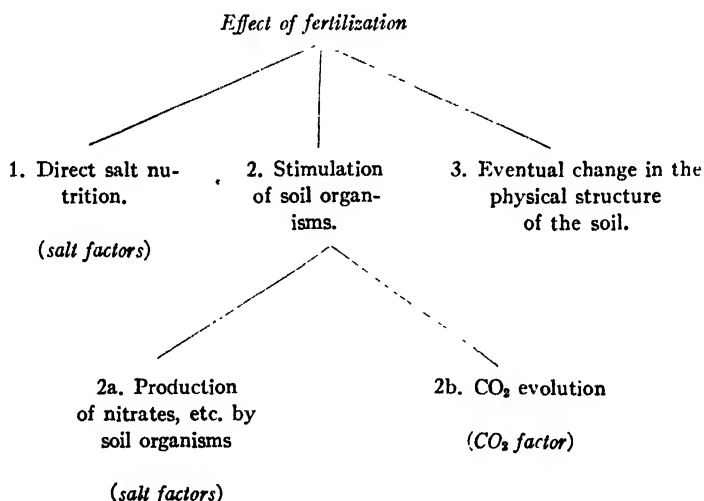
There is another fact that must be kept in mind in discussing the problem of indirect CO_2 fertilization. The fertilizer not only stimulates the microbiological activity in the soil but also acts directly as nutrition for the higher plants. This direct action is the only one to which attention has hitherto been paid in agriculture.

The growth of the plant is controlled by a number of growth factors, including carbon dioxide and the nutrient salts, the carbon dioxide very often controlling the yield. For the full utilization of the carbon dioxide, of course, a sufficient supply of nutrient salts is necessary. When the concentration of the salts on the other hand is too high, a retarding effect may result and this again will reduce the effect of the CO_2 factor. From this viewpoint a too heavy manuring or fertilizing, although it highly favors the soil respiration, is to be regarded as impractical. Only certain vegetables seem to endure very high quantities of

manure, and on the other hand it is a well known fact that beets require abundant salts.

There is another limit for the indirect CO_2 fertilization. It has been stated that the lower limit for the injurious effect of CO_2 accumulation in the pore space of the soil is about 1 per cent 15 cm. below the soil surface. When the CO_2 evolution is stimulated without provision for facilitating the aeration, the CO_2 concentration in the soil increases and finally the 1 per cent limit is attained and surpassed. As the normal concentration in 15 cm. depth reaches about 0.3 per cent, there is a broad margin for fertilization. Many plants endure CO_2 concentrations above 1 per cent.

The total effect of manure and mineral fertilizers is thus always split up into two chief components: first, the direct salt action upon the higher plants (salt factors), and, second, the indirect salt action (CO_2 factor), i.e., the stimulating effect upon the microörganisms. But these two chief actions are to a certain extent interwoven, for with stimulation of the soil activity, more nitrates etc. are produced (see following scheme).



On account of the circumstances mentioned, there will be in general a certain parallelism between soil respiration and crop yield, with fertilization experiments on the same soil. The soil respirations of different soils do not stand in any simple relation to the fertility: for example, a clay soil of low respiration may be more fertile than a sandy humus soil of high respiration (table 11). The microbiological population and the salt content of these two types of soils are too different to permit the use of the respiration as an indicator of the total fertility.

Figures 17 and 18 demonstrate a striking parallelism between soil respiration and yield. To what extent is the increase in yield due to the carbon dioxide factor? If we know the quotient Ak (p. 434), the part which the CO_2 factor

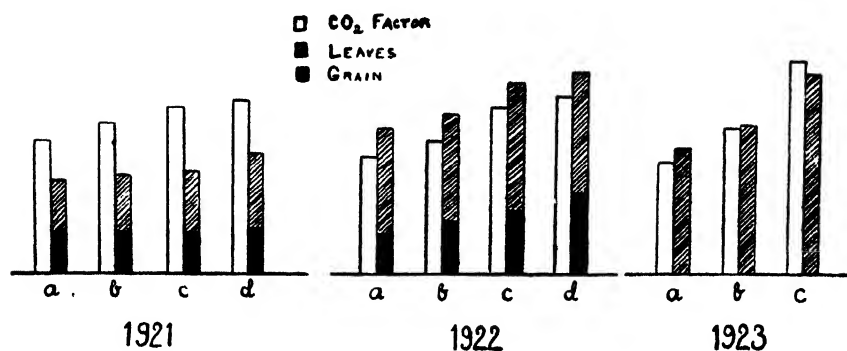


FIG. 17. DIAGRAM SHOWING THE PARALLELISM BETWEEN CARBON DIOXIDE FACTOR AND THE YIELD OF OATS

a = no manure, b = farmyard manure, c = mineral fertilizer, d = mineral fertilizer + manure.

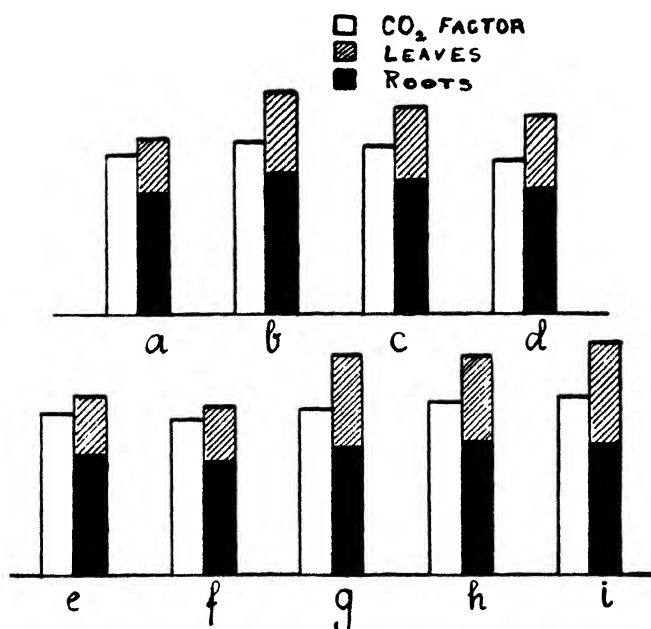


FIG. 18. DIAGRAM SHOWING THE PARALLELISM BETWEEN CARBON DIOXIDE FACTOR AND YIELD OF SUGAR BEETS

a-d = increasing intensities of hacking, e = no manure, f = farmyard manure, g = mineral fertilizer, h and i = mineral fertilizer + manure.

alone plays can be calculated. As already stated further experiments are necessary for estimating accurate values of Ak . It will probably develop that Ak varies also with the salt nutrition. Starting with Ak as low as 0.60

TABLE 13
Carbon dioxide factor and yield

<i>Oats, 1921</i>				
	(a) 300 KGM. MANURE HACKING	(b) 300 KGM. MANURE	(c) 400 KGM. MANURE	(d) 3 KGM. SUPER- PHOSPHATE, 6 KGM. POTAS- SIUM CHLORIDE, 4 KGM. SODIUM NITRATE
CO ₂ factor.....mgm. {	0.413	0.466	0.512	0.534
CO ₂ relative value	±0.028 77.7	±0.047 99.9	±0.040 108.7	±0.052 113.4
Yield {				
Seeds.....kgm.	12.5	12.5	12.07	13.17
Straw.....kgm.	16.0	17.75	19.76	21.16
S:a.....kgm. {	28.50	30.25	31.83	35.33
	±1.10	±0.60	±0.88	±1.05
<i>Oats, 1922</i>				
	(a) NO MANURE	(b) 300 KGM. MANURE	(c) 3 KGM. SUPER- PHOSPHATE, 3 KGM. POTAS- SIUM CHLORIDE, 3 KGM. SODIUM NITRATE	(d) 3 KGM. SUPER- PHOSPHATE, 3 KGM. POTAS- SIUM CHLORIDE, 3 KGM. SODIUM NITRATE, 300 KGM. MANURE
CO ₂ factor.....mgm. {	0.363	0.408	0.506	0.543
CO ₂ relative value	±0.019 68.5	±0.018 75.6	±0.019 94.2	±0.014 101.3
Yield {				
Seeds.....kgm.	31	32	42	43
Straw.....kgm.	11	16	20	23
S:a.....kgm. {	42	48	62	66
	±1.68	±2.45	±2.50	±2.51
<i>Oats, 1923</i>				
	(a) NO MANURE	(b) 3 KGM. SUPERPHOS- PHATE, 3 KGM. POTASSIUM CHLORIDE, 3 KGM. SODIUM NITRATE	(c) 3 KGM. SUPERPHOS- PHATE, 3 KGM. POTASSIUM CHLORIDE, 3 KGM. SODIUM NITRATE, 300 KGM. MANURE	
CO ₂ factor.....mgm. {	0.344	0.452	0.662	
CO ₂ relative value	±0.040 1.9	±0.034 81.3	±0.039 119.1	
Yield (total).....kgm. {	58.5	76.5	93.8	
	±2.50	±0.88	±1.25	

TABLE 13—*continued**Sugar beets,* 1925*

	(a) HACKING FOUR TIMES 5/22/5 TO 6/27/6	(b) HACKING SIX TIMES 5/22/5 TO 6/29/6	(d) HACKING THIRTEEN TIMES 5/22/5 TO 8/26/6
CO ₂ factor..... <i>mgm.</i>	0 6591	0.6398	0.5826
CO ₂ relative value	107.0	103 8	94.5
Yield roots† <i>kgm.</i>	219	206	191
Yield relative value	107 0	101.0	93.0
Yield leaves..... <i>kgm.</i>	124	117	115
S:a <i>kgm.</i> {	343	323	306
	107 0	101.0	95 5
Ratio, roots : leaves	1 77	1.76	1.66

	(c) NO MANURE	(f) 400 KGM. MANURE	(g) 4 KGM. SUPER- PHOSPHATE, 4 KGM. POTAS- SIUM CHLORIDE, 4 KGM. SODIUM NITRATE	(h) 4 KGM. SUPER- PHOSPHATE, 4 KGM. POTAS- SIUM CHLORIDE, 4 KGM. SODIUM NITRATE, 200 KGM. MANURE	(i) 4 KGM. SUPER- PHOSPHATE, 4 KGM. POTAS- SIUM CHLORIDE, 4 KGM. SODIUM NITRATE, 400 KGM. MANURE
CO ₂ factor <i>mgm.</i>	0 6130	0 6081	0 6204	0 6414	0 6539
CO ₂ relative value	100 0	99 6	101.1	104 9	106 95
Yield roots† <i>kgm.</i>	187	178	195	203	200
Yield roots relative value	100 0	95.0	104 3	108 6	107 0
Yield leaves..... <i>kgm.</i>	88	88	141	139	155
Total. <i>kgm.</i> {	275	266	336	342	355
	±15 6	±5 7	±6 4	±3 3	±5.9
Total relative value	100.0	96 7	122 3	124 3	129 0
Ratio, roots : leaves. . . .	1.59	1.73	1 28	1 34	1.29

* Plots a, b, and d fertilized with 3 kgm. superphosphate, 3 kgm. potassium chloride, 3 kgm. sodium nitrate to 100 square meters.

† To each 50 sq. m.

(p. 435) we nevertheless will find that in the case of oats the CO₂ factor constitutes 50 per cent or more of the total fertilizing effect (17, p. 278). In other cases, as for example sugar beets in 1925, the increase in yield due to the carbon dioxide is smaller in comparison to the high "salt effect." In plots a, b, d and where only the carbon dioxide factor was varied, the yield depends wholly upon it (table 13). In plot g, having the same carbon dioxide factor as plot e but received mineral salts, the yield increased 22.3 per cent. When by the addition of manure, the carbon dioxide factor is raised to 106.95, the

yield will rise in the same proportion, that is from 122.3 to 129.0. No doubt if it were possible to raise the carbon dioxide factor still more the yield would be correspondingly higher (table 9).

The soils in the oat experiments and in the sugar beet experiments were of a quite different quality. The former was a sandy loam rich in humus but poor in salts, the latter was a heavy clay soil not very rich in humus but rather fertile. Fertilizing with mineral salts, therefore, in the sandy loam will highly stimulate the carbon dioxide evolution and maintain a high CO_2 factor (see table 6 where an increase in carbon dioxide evolution up to 357 per cent is demonstrated). In the clay soil, the humus content probably limits the CO_2 evolution and the CO_2 factor, whereas the salt allows free development. The results indicate the importance of separating the two chief effects of fertilization according to the scheme on page 444.

For estimating the carbon dioxide factor that controls the plant growth, the direct analysis of the atmosphere among the assimilating leaves is accurate.² The measuring of the soil respiration serves well for comparing the effects of different treatments of the soil upon the carbon dioxide factor, but as the assimilation and the movements of the air among the leaves vary with the plants only the direct determination can indicate something about the prevailing CO_2 deficit or excess and the demands for fertilizing with this gas. It is obvious too that the CO_2 factor behaves differently from the salt factors with the variations of climate. The carbon dioxide is, for example dissipated by winds whereas the salts are washed out by rain. No two identical soils will therefore give the same yield, if the wind exposition is different.

REFERENCES

- (1) BARTHEL, CHR. 1923 Action of manure upon the decomposition of cellulose in the soil. Beretning fra N. J. F.: Kongress, June 1923. *Nordisk Jordbrugsforskning*, p. 299, Copenhagen.
- (2) CLEMENTS, F. E. 1921 Aeration and air content. Carnegie Inst. Wash. Pub. 315.
- (3) CONN, H. J. 1910 Bacteria in frozen soil. *Centbl. Bakt.* 28: 422-434; 42: 510-519.
- (4) DELF, E. M. 1915 *New Phytol.* 14: 63.
- (5) FODOR, J. v. 1882 Hygienische Untersuchungen über Luft, Boden und Wasser, v. 2. Wien.
- (6) GAINES, P. L. 1919 Parallel formation of carbon dioxide, ammonia, and nitrate in soil. *Soil Sci.* 7: 293-311.
- (7) HANSTEEN-CRANNER, B. 1922 Zur Biochemie und Physiologie der Grenzschichten lebender Pflanzenzellen. *Meld. Norges Landbr. Høiskole* 2: 1.
- (8) HESSELMAN, HENRIK. 1910 The oxygen in soil water and growth of forest. *Meddel. Statens Skogsforsöksans.* (Sweden) 7: 91.

² As the CO_2 concentration is not the same at the level of the upper leaves as among the basal leaves, determinations from the middle of the leaf mass will give only an average, which, nevertheless, is sufficient for our purpose. It would be a very difficult task to make single determinations for each leaf, and such detailed work has no great value since the light factor varies from leaf to leaf. Moreover, the drawing of a 2.3 liter sample in a few minutes will give a very good idea of the average concentration among the whole leaf mass, when this is not too extended in vertical direction.

- (9) HILTNER, L. 1904 Recent experiments and new problems in soil bacteriology, with especial reference to green manuring and fallows. *Arb. Deut. Landw. Gesell.*, 98: 69.
- (10) KÖNIG, J., AND HASENBÄUMER, J. 1921 Die Bedeutung neuer Bodenforschungen für die Landwirtschaft. *Landw. Jahrb.*, 55: 185-252.
- (11) LAU, E. 1906 Beiträge zur Kenntnis der im Ackerboden befindlichen Luft. Dissertation, Rostock.
- (12) LEATHER, J. W. 1915 Soil gases. *Mem. Dept. India. Pusa., Chem. Ser.*, 4: 85-134
- (13) LOHNIS, F., AND SABASCHNIKOFF, A. 1908 Ueber die Zersetzung von Kalkstickstoff und Stickstoffkalk. *Centbl. Bakt.* (II) 20: 322-332.
- (14) LUNDEGÅRDH, H. 1922 Neue Apparate zur Analyse des Kohlensäuregehalts der Luft. *Biochem. Ztschr.* 131: 109.
- (15) LUNDEGÅRDH, H. 1923 Die Bedeutung des Kohlensäuregehalts und der Wasserstoffionenkonzentration des Bodens für die Entstehung der Fusariosen. *Bot. Notiser*, p. 25.
- (16) LUNDEGÅRDH, H. Über die Kohlensäureproduktion und die Gaspermeabilität des Bodens. *Arkiv. Bot.* 18: (13).
- (17) LUNDEGÅRDH, H. 1924 Der Kreislauf der Kohlensäure in der Natur. Ein Beitrag zur Pflanzenökologie und zur landwirtschaftlichen Düngungslehre, Jena.
- (18) LUNDEGÅRDH, H. 1925 Der Temperaturfaktor bei Kohlensäure assimilation und Atmung. *Biochem. Ztschr.* 154: 195.
- (19) LUNDEGÅRDH, H. 1925 Klima und Boden in ihrer Wirkung auf das Pflanzenleben. Jena.
- (20) MELIN, E. 1925 Untersuchungen über die Bedeutung der Baummykorrhiza. Eine ökologisch-physiologische Studie. Jena.
- (21) MITSCHERLICH, A. 1920 Bodenkunde für Land- und Forstwirte, ed. 3. Berlin.
- (22) MUNTZ, A., AND GAUDECHON, H. 1912 The awakening of the soil. *Compt. Rend. Acad. Sci. (Paris)*, 154: 163-168.
- (23) NELLER, J. R. 1918 Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *Soil Sci.* 5: 225-242.
- (24) NIENBURG, W. 1923 Handbuch der Binnenfischerei Mitteleuropas, v. 1, p. 85.
- (25) PETERSON, P. 1871 Ueber den Einfluss des Mergels auf die Bildung von Kohlensäure und Salpetersäure in Ackerboden. *Landw. Vers. Sta.* 13: 155-175.
- (26) PETTENKOFER, M. Wollnys Forschungen Agrikulturphysik, v. 5, p. 300.
- (27) POTTER, R. S., AND SNYDER, R. S. 1916 Carbon dioxide production in soils and carbon and nitrogen changes in soils variously treated. *Iowa Agr. Exp. Sta. Res. Bul.* 39.
- (28) ROMELL, L. G. 1922 Soil ventilation as an ecological factor. *Meddel. Statens Skogsforsöksanst.* 19: 125-359.
- (29) RUSSELL, E. J., AND APPLEYARD, A. 1915 The atmosphere of the soil: its composition and the causes of variation. *Jour. Agr. Sci. (England)* 7: 1-48.
- (30) RUSSELL, E. J. 1921 Soil conditions and plant growth, ed. 4. London.
- (31) SCHRODER, H. 1919 *Naturwissenschaften* 7: 8.
- (32) STOKLASA, J., AND ERNEST, A. 1905 Ueber den Ursprung, die Menge und die Bedeutung des Kohlendioxyds im Boden. *Centbl. Bakt.* (II) 14: 723-736.
- (33) SUCHTELEN, H. VAN 1910 Über die Messung der Lebenstätigkeit der äobiotischen Bakterien im Boden durch die Kohlensäureproduktion. *Centbl. Bakt.* (II) 28: 45-89.
- (34) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Microbiological analysis of soil as an index of soil fertility: VII. Carbon dioxide evolution. *Soil Sci.* 17: 141-161.
- (35) WILSON, J. K. 1920 Device for growing large plants in sterile media. *Phytopath.* 19: 425-429.
- (36) WOLLNY, E. 1880 Untersuchungen über den Kohlensäuregehalt der Bodenluft. *Landw. Vers. Sta.* 25: 273-391.

PLATE 1

FIG. 1. Arrangement for determination of the Soil Respiration. To the left, the "respiration bell;" to the right, portable apparatus for CO_2 determination; connected by a rubber tube.

FIG. 2. The experiment field in 1925 with field laboratory and apparatuses for CO_2 determination, one on each plot.

FIG. 3. Interior of the field laboratory, showing the analysis apparatus at the left, the portable apparatus and respiration bell at the right.



FIG. 1



FIG. 2



FIG. 3

PLATE 2

FIGS. 1 AND 2. Sugar beets from the non-gassed glasshouse 1. Carbon dioxide concentration 0.6806 mgm. to each liter of air.

FIGS. 3 AND 4. Sugar beets from the gassed house 2. Carbon dioxide concentration 0.7705 mgm. to each liter of air.



FIG. 1



FIG. 3



FIG. 4

FIG. 2

THE SEEDLING PLANT METHOD OF DETERMINING SOIL NUTRIENT DEFICIENCY

J. W. AMES AND R. W. GERDEL

Ohio Agricultural Experiment Station

Received for publication July 10, 1926

Since the beginning of agricultural research, the problem of determining plant nutrient deficiencies in the soil has been of major importance. Various methods of solving this problem have been proposed, including a complete analysis of the soil, extraction by acids of various kinds and strengths as well as by physiological means. It must be admitted, however, that at the present time there is no known method which is both accurate and generally adaptable.

During recent years, the use of growing plants as a means of obtaining indications of nutrient availability has received rather wide attention, to some extent in this country, but more extensively in continental Europe.

Mitscherlich (14) in Germany, has proposed a method of pot culture, involving the additions of complete fertilizers and of fertilizers deficient in the element under investigation. He has evolved a mathematical equation which when applied to field and pot yields is believed to indicate the deficiency of any given nutrient.

Holben and Haley (11) at the Pennsylvania Station, working with buckwheat plants, found some correlation between acid-soluble potash of the soil and potash removed by the plant.

Hoffer (10) of the Indiana Station has recently reported a method of analyzing the corn plant, both micro and macrochemically, which he claims gives an indication of the supply of nitrates and available potash in the soil.

The biological method of soil analysis proposed by Neubauer (15, 16) has been rather thoroughly investigated abroad, and has received some attention in this country, particularly by the sugar industry (6, 17). This method has been previously described by the authors (1).

As several foreign investigators (2, 4, 5, 12, 13, 18, 19) have reported upon the practical application of the seedling plant method for determining the nutritive requirements of the soil, it seemed advisable to investigate the practical application of this method as an indicator of the fertility requirements of soils in this country.

EXPERIMENTAL

As previously reported (1) it was found necessary to modify the original Neubauer procedure. Hahne (8, 9) has also found modification essential to good results.

It was found advisable to use larger pots of 12½ cm. diameter and 7 cm. depth mainly to give sufficient area to prevent undue crowding of the seedlings. A dilution of 200 gm. of soil and 1000 gm. sand was found to give best results. As the results with rye were unsatisfactory, wheat and several other plants, including corn, buckwheat, and soybeans have been tried.

For satisfactory results in a test of this kind it is necessary to choose seed with a high percentage of germination and uniformity in composition, for it is essential that the seedlings make an even and rapid growth.

Soils from the 5-year rotation experiment of the Ohio Agricultural Experiment Station at Wooster were used in this series of tests. A few sandy soils from the Lake Erie district were also included for comparison, since these sandy soils are commonly considered to be deficient in potassium.

Series 1 consists of plot soils that have received various combinations of fertilizers. As the soils of these plots have been under regular treatment for a period of 30 years, it would be expected that some would be markedly deficient in the element or elements not included in the fertilizer treatment received.

The selected wheat seeds, previously treated with "Upsulun," a chlorphenol mercury compound, were planted, 100 to a pot, in the soil previously made up as above and brought to optimum moisture content. At the end of 18 days the tops and roots were harvested separately and analyzed for phosphorus and potassium.

The object of making a separate analysis of the roots, rather than using the whole plant, as Neubauer and others have done, was to determine whether the tops are as good indicators of nutrient availability as the whole plant, since if they should be, the error due to inability thoroughly to remove the soil from the roots, and to secure all the roots in the pot would be avoided.

The data presented in tables 1 and 5 indicate that it is unnecessary to analyze the roots. The potassium assimilation by the tops has in every case paralleled the assimilation by the whole plant.

Comparison of potassium and phosphorous assimilation by pot and field samples

The wheat series were planted in pots late in September on the same date that the wheat was sown in the field. At the end of 18 days when the pots were harvested, representative samples from each field plot were also harvested and analyzed for potassium and phosphorous. By growing the pot series through the same period of time it was hoped to eliminate as far as possible certain errors between field and pot samples, particularly photo-periodic effects. The analyses of the field samples were calculated to the same weight as the plants in the pot test. As shown in table 2, representing soils of the 5-year rotation, and table 6, representing soils of the 3-year rotation (20), the potassium assimilated in pot culture varied directly with that assimilated in the field in every case excepting plot 11 of the 5-year rotation. This is the complete fertilizer plot. Apparently the pot cultures are fair indicators of field conditions,

TABLE 1

Relative value of wheat tops, roots, and total plant material as indicators of potassium and phosphorous absorption

(Soils from 5-year rotation)

PLOT NUMBER	TREATMENT	WEIGHT OF PLANTS			POTASSIUM ABSORBED			PHOSPHORUS ABSORBED		
		Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total
		gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
	Pure sand	0.954	1.648	2.602	7.35	6.55	13.90	9.40	7.38	16.78
2	P ₂ O ₅	1.162	1.543	2.705	10.85	7.07	17.92	9.74	6.47	16.21
3	KCl	1.219	1.605	2.824	14.11	8.64	22.75	9.94	6.57	16.41
5	NaNO ₃	1.122	1.472	2.594	10.24	7.77	18.01	9.54	6.77	16.31
6	P ₂ O ₅ + NaNO ₃	1.160	1.510	2.660	11.25	7.43	18.68	8.86	6.77	15.63
8	KCl + P ₂ O ₅	1.155	1.409	2.564	14.31	10.41	24.72	8.99	6.16	15.15
9	KCl + NaNO ₃	1.207	1.460	2.667	12.75	8.72	21.49	9.03	7.24	16.27
11	KCl + NaNO ₃ + P ₂ O ₅	1.198	1.499	2.697	11.70	7.75	19.45	8.36	8.73	17.09

TABLE 2

Comparison of wheat yield and acid-soluble potassium with the available potassium by the Neubauer test and that present in field samples—tops only used as indicators

(Soils from 5-year rotation, Wooster)

PLOT NUMBER	WHEAT YIELD	ACID-SOLUBLE K	AVAILABLE K BY NEUBAUER TEST	K IN FIELD SAMPLE
	bu.	p.p.m.	mgm.	mgm.
2	22.0	17	10.85	18.49
3	14.88	36	14.11	26.72
5	14.30	23	10.24	14.15
6	25.88	17	11.25	13.72
8	24.49	30	14.31	23.09
9	15.32	32	12.75	21.56
11	30.33	31	11.70	28.10

Field samples based on same weight as 100 plants from pot tests.

TABLE 3

Comparison of phosphorus extraction by "Neubauer method" with actual assimilation in the field and the yield of wheat

(Soils from 5-year rotation, Wooster)

TEST	PLOT 2	PLOT 3	PLOT 5	PLOT 6	PLOT 8	PLOT 9	PLOT 11
Neubauer method, mgm.....	9.74	9.94	9.54	8.86	8.99	9.03	8.36
Field sample, mgm.	8.26	6.47	5.56	6.74	7.35	4.68	7.43
Wheat yield, bu.....	22.0	14.88	14.30	25.88	24.49	15.32	30.33

Field samples based on weight of 100 plants in pot test.

TABLE 4

Comparison of phosphorus extraction by "Neubauer method" with actual assimilation in the field
(Soils from 3-year rotation, Wooster)

TEST	PLOT 2	PLOT 3	PLOT 5	PLOT 6	PLOT 8	PLOT 9	PLOT 11
Neubauer method, <i>mgm.</i>	9.44	10.58	9.27	7.75	8.08	8.63	7.48
Field sample, <i>mgm.</i>	7.41	6.32	5.87	6.10	7.50	4.90	6.86
Wheat yield, <i>bu.</i>	34.82	28.61	27.84	34.74	35.97	30.13	36.82

TABLE 5

Relative value of tops, roots, and total plant material as indicators of potassium and phosphorus absorption
(Soils from 3-year rotation)

PLOT NUM- BER	TREATMENT	WEIGHT OF PLANTS			POTASSIUM ABSORBED			PHOSPHORUS ABSORBED		
		Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
	Pure sand	1.033	1.531	2.564	6.23	7.91	14.14	8.76	8.19	16.95
2	P ₂ O ₅	1.061	1.413	2.474	9.20	9.08	18.28	9.44	8.09	17.53
3	KCl	1.305	1.364	2.669	16.56	10.72	27.28	10.58	8.52	19.10
5	NaNO ₃ + dried blood	1.173	1.539	2.712	9.76	8.88	18.64	9.27	8.73	18.00
6	NaNO ₃ + P ₂ O ₅ + dried blood	1.127	1.570	2.697	8.92	9.96	17.88	7.75	6.53	14.28
8	P ₂ O ₅ + KCl	1.254	1.532	2.786	14.39	9.12	23.51	8.08	5.62	13.70
9	NaNO ₃ + KCl + dried blood	1.219	1.539	2.758	10.60	9.68	20.28	8.63	7.54	16.17
11	NaNO ₃ + P ₂ O ₅ + KCl + dried blood	1.061	1.442	2.503	8.48	8.68	17.16	7.48	6.81	13.29

TABLE 6

Comparison of wheat yield and acid-soluble potassium with the available potassium by Neubauer test and that present in field sample—tops only used as indicators
(Soils from 3-year rotation, Wooster)

PLOT NUMBER	WHEAT YIELD	ACID-SOLUBLE POTASSIUM	K BY NEUBAUER TEST	K IN FIELD SAMPLE
	<i>bu.</i>	<i>p.p.m.</i>	<i>mgm.</i>	<i>mgm.</i>
2	34.82	25	9.20	24.35
3	28.61	37	16.56	48.48
5	27.84	28	9.76	23.75
6	34.74	22	8.92	16.59
8	35.97	..	14.39	39.11
9	30.13	..	10.60	34.67
11	36.82	..	8.48	30.50

although plants grown in the field assimilated much greater quantities of potassium, probably because of the larger amount of soil at their disposal.

The converse is true of phosphorous assimilation, as shown in tables 3 and 4. In every case the field-grown plants contain less phosphorous than those of the pot cultures, and furthermore, the pot cultures are remarkably uniform in phosphorous assimilation, showing much less variation than the field samples. Apparently the pot cultures give little indication of field nutritional conditions so far as phosphorous is concerned.

Comparison of potash assimilation in pot culture with the soluble potash of the soil and with the wheat yield

There are wide differences in the centi-normal nitric-acid-soluble potassium in these soils as shown by Ames and Simon (2). A comparison of the potassium assimilated by wheat plants in pot culture with the acid-soluble potassium of the soil shows practically no correlation for the soils from plots of the 5-year

TABLE 7
Results of Neubauer test with wheat seedlings on Dunkirk and Maumee fine sands

SOIL	PREVIOUS TREATMENT	ACID-SOLUBLE K IN SOIL	POTASSIUM ABSORBED		
			Tops	Roots	Total
		<i>p p.m.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Dunkirk fine sand	Virgin	36.27	11.21	8.20	19.41
Dunkirk fine sand	Cropped	53.10	11.94	8.68	20.62
Dunkirk fine sand	Potatoes, then pas- ture 15 years	32.78	10.20	8.65	18.85
Maumee fine sand	Virgin	14.48	12.58	7.47	20.05

rotation. In the case of the 3-year rotation fertility soils there appears to be some agreement for the first four plots, although the lack of acid-soluble figures for the last three soils of this series precludes drawing any definite conclusions.

Comparison of wheat yield with potash assimilated by wheat seedlings

A comparison of the average wheat yield for the last five years on these plots, with the available potassium as indicated by the seedlings, is given in tables 2 and 6. It is notable that plot 11 of both the 3 and 5-year rotations, gives the highest yield, as would be expected since these plots received a complete fertilizer treatment. The biologically available potassium on these plots, however, is much less than for plot 9 of both rotations, which received only nitrates and potash and produced a much lower average yield, whereas the assimilated potassium for plot 11 is but slightly greater than from plot 6 for both rotations, although the latter plot has never received any addition of potassium.

An explanation of this may be that the heavy yields of plot 11 have tended to utilize the available potassium to such an extent that there has been no ac-

cumulation in the soil. From the results for those plots which have never received potassium additions; namely, plots 2 and 6 of both rotations, it would appear that the yields have been so low that the supply of native potash becoming available in these soils has not been greatly reduced.

As a further check upon the reliability of this method, particularly the use of the wheat plant, a series was grown, using Dunkirk and Maumee fine sands from the Lake Erie district. A description of the soils with the results obtained is given in table 7.

Here again it is to be noted that the tops are better indicators of potassium availability than is the whole plant, since the tops exhibit considerable variability, whereas the roots are remarkably uniform in potassium content.

Although these sandy soils are considered to be naturally deficient in potassium, the assimilated potassium is in every case equal to, or more than, that from plots in the 5- and 3-year rotations which have received no potassium. The acid-extractable potassium is also remarkably high upon these soils and there is no correlation between the acid-soluble and the biologically available potassium.

Phosphorous availability

The data in tables 1, 3, 4, and 5, show that in no case is the variation in the phosphorous assimilation by the wheat seedlings of sufficient magnitude to indicate any variation in availability. In fact the data in tables 1 and 5 show that the phosphorous assimilated by the plants corresponds closely to the amount in the seed, the phosphorous content of plants in the sand checks being in most cases equal to or greater than that of plants grown on diluted soil. On the other hand, the phosphorous content of plants grown in the field varies as much as 3 or 4 mgm. among the different plots.

Studies with other plants

Although the variation in potassium assimilation indicates that the seedling test may have some value, the results for phosphorous show that the wheat plant can not be used as an indicator of the availability of this element. In order to determine whether other plants than wheat might have greater utility, three new series using corn, soybeans, and buckwheat were grown in soils from the same plots used for the wheat series.

These plants were grown 18 days and since results from wheat plants had indicated that it was unnecessary to utilize the roots, only the tops were harvested.

The buckwheat series proved of no value. The germination percentage was very low and extremely variable; the seedlings were very spindling and many cotyledons had dropped off, leaving only a bare, leafless stem by the eighteenth day.

The results of analyses of corn for potash and phosphorous are given in table 8. Although there is a considerable variation in the assimilated potas-

sium, there is no direct correlation with the acid-soluble potassium or yield of the plots other than that an increased yield seems to have greatly reduced the available potassium of the soil even with a considerable addition of potash salts. The reduction is greater than on soils receiving no potash additions and having, of course, much lower corn yields. Apparently the potassium content of the Dunkirk and Maumee fine sands is not so readily assimilated by corn seedlings as by wheat seedlings (table 10).

The phosphorous assimilation is no greater and shows no larger variation than in the wheat series, there being no appreciable assimilation above the seed content as shown by the silica sand check. In many cases the soil cultures fall below the check plots.

Although in a previous test (1), corn appeared to be a better indicator than wheat of potassium availability, the data for this series do not corroborate the

TABLE 8

Comparison of potassium and phosphorous assimilation by the corn plant using the Neubauer method, with acid-soluble potassium and crop yields

PLOT NUMBER	SOIL TREATMENT	K IN SOIL	CORN YIELD 1919-1923	K ASSIMILATED	P ASSIMILATED
		p p.m.	bu	mgm.	mgm.
1	None	23	33.25	14.19	13.65
2	P ₂ O ₅	17	34.48	12.14	13.85
3	P ₂ O	36	47.16	18.49	14.09
8	P ₂ O ₅ + K ₂ O	30	53.71	12.94	13.78
11	P ₂ O ₅ + K ₂ O + N	31	58.67	10.57	13.45
	Dunkirk, Virgin	36	9.96	13.04
	Dunkirk, Crop Experiment	53	9.76	11.93
	Dunkirk, potato then pasture 15 years	32	9.65	12.20
	Maumee Virgin	14	10.73	11.32
	Silica sand check	8.96	12.67

former results. The previous test involved only 5 plants, whereas this series was run with 25 plants to the pot more nearly to attain a starvation condition as advised by Neubauer (15, 16). It is probable, therefore, that the favorable results obtained previously were due to more nearly optimum growth factors than are provided for in the seedling method as devised by Neubauer.

A soybean series was also grown, 25 seeds being used to the pot, as for corn. The results which are of particular interest are given in table 9. The potassium assimilation does not vary in the same direction as for corn and wheat.

The higher potassium assimilation of soybeans follows the fertilizer treatment. It will be noted that the potassium assimilation is larger from the complete fertilizer plot and the plot that has had addition of phosphorous and potassium. Soybeans as compared with wheat and corn have also indicated a greater potassium availability for the sandy soils.

Although it may appear from these results that the soybean is more satisfactory than the other plants for indicating variations in potassium availability,

TABLE 9
Comparison of potassium and phosphorus assimilation by soybeans with the acid-soluble potassium in soil

PLOT NUMBER	SOIL TREATMENT	ACID-SOLUBLE K IN SOIL	K ASSIMILATED	P ASSIMILATED	P IN SEED*
		<i>p.p.m.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1	None	23	35.62	13.11	12.68
2	P ₂ O ₅	17	30.80	9.50	10.62
3	K ₂ O	36	36.67	14.09	12.39
8	P ₂ O ₅ + K ₂ O	30	39.28	14.32	14.46
11	P ₂ O ₅ + K ₂ O + N	31	45.43	15.88	14.46
	Dunkirk-Virgin	36	39.68	11.21	13.57
	Dunkirk, cropped	53	44.31	15.30	14.75
	Dunkirk, potatoes, then pasture 15 years	32	40.25	13.72	14.16
	Maumee-Virgin	14	47.37	15.67	14.46
	Silica sand check	..	35.42	14.46	14.46

* Calculated from sand check.

TABLE 10
*Comparison of potassium assimilated in excess of pure sand checks by wheat, corn, and soybeans**

SOIL SOURCE	POTASSIUM ASSIMILATED IN EXCESS OF SAND CHECK		
	Wheat	Corn	Soybeans
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Plot 1	1.20	5.23	4.60
Plot 2	3.50	3.18	4.78
Plot 3	6.76	9.53	6.43
Plot 5	2.89
Plot 6	3.90
Plot 8	6.96	3.98	4.00
Plot 9	5.40
Plot 11	4.35	1.61	10.15
Dunkirk-Virgin	3.86	1.00	6.56
Dunkirk-Cropped	4.59	0.80	9.03
Dunkirk-Pasture	2.85	0.69	5.69
Maumee-Virgin	5.23	1.77	12.09

* Because the soybeans are very high in potassium and the cotyledons were included in the analysis and since the number of soybean plants to the pot varied considerably, the values are here calculated to the number of plants to the pot.

it is not a suitable plant for the seedling test. Soybean seed has a high potassium content, and the possibility of wide differences in the amount in different lots of 25 seeds, together with the inability to secure a consistent germination

percentage renders the interpretable value of the data doubtful. It is apparent that the phosphorus assimilation does not vary consistently, nor is there a noticeable excess beyond that of the pure sand check.

Table 10 consists of the condensed data of these three series. In each case the potassium content of the pure sand check has been subtracted from the remainder of the pots in the series in order more closely to compare the data. In the case of soybean, where the number of plants to the pot varied from 18 to 25, the value of a single plant grown on pure sand was determined and this value multiplied by the number of plants in each pot before subtraction. The variation for the three crops is shown graphically in figure 1. The value

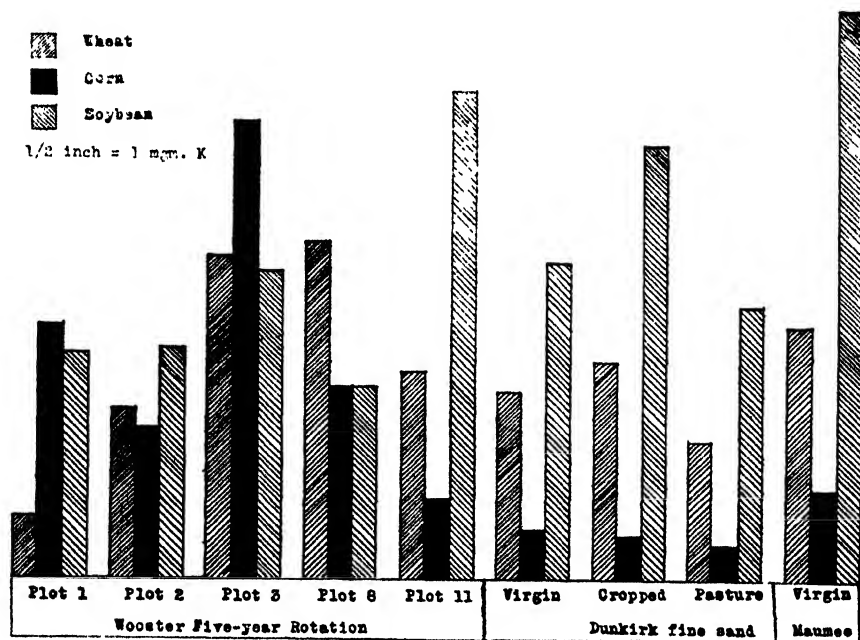


FIG. 1. POTASSIUM ASSIMILATED BY DIFFERENT CROP PLANTS

of these data consists largely in the evidence produced that no one plant, such as rye, used by Neubauer can serve as an indicator of the availability of any given nutrient for all plants. There exists no consistent relation between the assimilation of potassium by the three plants, corn, wheat, and soybeans upon the same soil.

Comparison of results with those obtained by Neubauer

Since Neubauer (16) has grown his rye seedlings on soils which for 30 years were under a system of fertilizer treatment similar to that given for an equal period to the soils used in the present study, a comparison of the two sets of data may be made as shown in table 11.

Although the data referring to the present study are for tops only, even should a reasonable allowance be made for the potassium in the roots, the results would in all cases still be only one-fifth to one-tenth as large as Neubauer's. There is also no evidence of correlation between the plot treatment and the potash assimilation of the two series of soils. In general, it

TABLE 11
Comparison of results with those obtained by Neubauer

KIND OF FERTILIZER USED	PRESENT STUDY—K ABSORBED BY			RESULTS OF NEUBAUER—K ABSORBED BY RYE
	Wheat	Corn	Soybeans	
	mgm.	mgm.	mgm.	
Unfertilized	1.20	5.23	4.60	29.1
P ₂ O ₅ only	3.50	3.18	4.78	26.2
K ₂ O only	6.76	9.53	6.43	56.0
N only	2.89	21.7
P ₂ O ₅ + NaNO ₃	3.90	25.5
P ₂ O ₅ + KCl	6.96	3.98	4.00	61.9
KCl + NaNO ₃	5.40	57.5
KCl + NaNO ₃ + P ₂ O ₅	4.35	1.61	10.15	59.4

TABLE 12
Variation in potassium absorption within the duplicates of a single series—corn series

POT NUMBER	SOIL	* NUMBER OF PLANTS	WEIGHT OF PLANTS	POTASSIUM ASSIMILATED	DIFFERENCE IN WEIGHT
			gm.	mgm.	mgm.
1	1	25	1.6610	14.31	
2		25	1.5341	14.07	0.24
3	2	25	1.5082	13.75	
4		25	1.2784	10.53	3.22
5	3	25	1.5790	15.92	
6		25	1.6116	21.07	5.15
7	8	25	1.5440	13.02	
8		25	1.5697	12.86	0.16
9	11	25	1.5154	10.21	
10		23	1.1038	10.93	0.72

must be said that the small absolute values for potash assimilation obtained in the present study, and the relatively large errors involved due to the variation between duplicate pots in a series render the value of the method very doubtful as applied to soils of the character studied.

The variation between duplicate pots, which are often as great as that between two different soils, can be explained only on a physiological basis, since

the weights of the plants grown in duplicate pots are remarkably uniform for wheat and corn. It is probable that a series of physiological factors involving inheritance and environment may be involved and these, in such an experiment as this, are beyond control. An attempt to control heritable variations was made, particularly in the case of corn, by using Burr Leaming, a double cross of inbred strains. Wheat and soybeans, being naturally inbred, should be fairly homozygous for growth factors within a given variety, though undoubtedly a pure line selection would be better than a variety.

Environmental factors might be better controlled by the use of artificial light and a constant temperature case. Neubauer (15, 16), however, has indicated that light is not an important factor, and Gunther (7) has confirmed this statement since he found but slight variation in potassium and phosphorous assimilation when the light was varied from complete darkness to full daylight. Wiessman (21) apparently disagrees with this, since he states that the stronger the light the greater is the influence upon seedling growth and nutrient assimilation.

Gunther (7) has also shown that the hydrogen-ion concentration of the soil does not materially influence the assimilation of nutrients by the seedling. This seemed to hold true in the present study, where there was no apparent relation of assimilation to the pH value of the soils used.

CONCLUSIONS

1. An investigation of the seedling plant method of determining the available soil nutrients indicates that the method devised by Neubauer is not generally applicable.
2. No one plant has been found which will indicate availability for all kinds of crop plants.
3. The error between duplicate pots is often of the same magnitude as the difference between the various soils under investigation.
4. Variations in potassium absorption were neither large enough nor sufficiently consistent to serve as a guide to the potassium requirements of the soils studied.
5. Contrary to Neubauer's results, no variation in phosphorous assimilation was found.
6. It may be possible so to standardize the seedling plant method that it may serve as an index of availability but it is believed that physiological limitations may exist which will be found insurmountable.

REFERENCES

- (1) AMES, J. W., AND GERDEL, R. W. 1927 Potassium content of plants as an indicator of available supply in soil. *Soil Sci.* 23: 199-223.
- (2) AMES, J. W., AND SIMON, R. H. 1924 Soil potassium as affected by fertilizer treatment and cropping. *Ohio Agr. Exp. Sta. Bul.* 379.
- (3) DENSCH, A. 1925 Wie lasst der Nahrstoffbedart unserer Boden ermitteln? *Illus. Landw. Ztg.* 6: 2-3.

- (4) DENSCH, A. 1926 Erfahrungen mit der Methode Neubauer. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 5: 97.
- (5) DENSCH, A. 1926 Die Bestimmung des Düngerbedürfnisses des Bodens durch Laboratoriumsversuche nach Neubauer. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 5: 130.
- (6) Editorial 1925 The Neubauer test. *Facts About Sugar* 20: 656.
- (7) GUNTHER, E. 1926 Kritisches Untersuchungen über die Keimpflanzenmethode von Neubauer. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 5: 32-36.
- (8) HAHNE, H. 1925 Untersuchungen über die Neubauersche Methode zur Bestimmung der wurzellosen Nährstoffe Phosphorsäure und Kali. Ungedr. Diss. Jena.
- (9) HAHNE, H. 1926 Die Neubauersche Methode zur Bestimmung der wurzellosen Nährstoffe Phosphorsäure und Kali. *Ztschr. Pflanzenernähr. u. Düngung*. (A) 6: 238-248.
- (10) HOFFER, G. N. 1926 A simple test for detecting the nutrient needs of the corn plant. (Abs.) *Jour. Amer. Soc. Agron.* 18: 29-31.
- (11) HOLBEN, F. J., AND HALEY, D. E. 1925 A biological measurement of the available potassium in soils. *Pa. Agr. Exp. Sta. Bul.* 196.
- (12) LEMMERMAN, O. 1926 Die Bestimmung des Düngungsbedürfnisses des Bodens durch Laboratoriumsversuche. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 5: 133 and 105.
- (13) MITSCHERLICH, E. A. 1926 Bemerkungen zu der Methode Neubauer. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 5: 132.
- (14) MITSCHERLICH, E. A. ET AL. 1923 Plant physiological solution of chemical soil analysis. *Landw. Jahr.* 58: 601-617; *Exp. Sta. Rec.* 50: 118.
- (15) NEUBAUER, H. 1924 The utilization of seedlings in the estimation of soil nutrients. *Internatl. Rev. Sci. and Pract. Agr. (Rome)* 2 (n. s.): 788-797.
- (16) NEUBAUER, H., AND SCHNEIDER, W. 1923 Die Nährstoffaufnahme der Keimpflanzen und ihre anwendung auf die Bestimmung des Nährstoffgehalts der Boden. *Ztschr. Pflanzenernähr. u. Düngung*. (A) 2: 329-341.
- (17) ROEMER, T. 1925 The Neubauer test for soil phosphate requirements. (Abs. and Eng. Transl.) *Facts About Sugar* 20: 400.
- (18) ROEMER, T. 1925 Zuckerrubensbau 7: 1.
- (19) ROEMER, T., DIRKS, O., AND GUNTHER, E. 1923 Neubauer-Analysen im Vergleich zu Felddüngungsversuchen. *Illus. Landw. Ztg.* 44: 1.
- (20) THORNE, C. E. 1924 The maintenance of soil fertility. *Ohio Agr. Exp. Sta. Bul.* 381.
- (21) WIESSMANN, H. 1925 Ueber den Einfluss des Lichtes auf die Nährstoffaufnahme der Pflanzen im Jugendstadium. *Ztschr. Pflanzenernähr. u. Düngung*. (B) 4: 153.

THE POSSIBLE RÔLE OF IRON DEPOSITING BACTERIA IN THE FORMATION OF HARD-PAN

C. S. MUDGE

University of California

Received for publication August 2, 1927

The presence of a layer of impervious material beneath the surface of a soil is usually a limiting factor in plant growth. True, there are instances where agricultural plants grow with extraordinary success in regions where a sub-surface stratum of this character is known to exist, but usually such a region is considered to offer little prospect of a continuously successful agricultural history. Hard-pan is a layer of just this sort. It is found as a calcarious pan, as well as one in which iron salts predominate. Of this latter, California has a considerable area, since of the 23,000,000 acres of arable land in the state, 2,000,000 acres are known, from soil surveys, to have an iron hard-pan below the surface. As hard-pan obstructs the penetration of water and roots, in the semi-arid conditions such as in California where roots of most agricultural plants penetrate to a considerable depth, blasting is often resorted to in an attempt to overcome the disadvantages of the pan.

Many explanations of the cause of hard-pan have been made in the past. That suggested by Hilgard (5) attributes the formation to a solution of materials at the weathering surfaces with a subsequent descent of these solutions to a lower level where the penetration of air "may cause the accumulation of the dissolved matter at a certain level year after year." But such a statement is, in fact, an empiricism and offers little satisfaction when the real explanation of the cause of pan formation is desired. Perhaps one of the most generally accepted of the theories is that which assumes successive stages of reduction and oxidation of the iron salts by the organic acids to a ferrous state with the subsequent precipitation of ferric salts through the action of air. Such a view is perhaps a little more definite than the older theories. Still more recently, however, attention has been directed toward the behavior of the colloids in the soil. With such newer knowledge, a theory has been developed by Morison and Sothers (7), who explain the pan formation by showing that in soils generally there is a favorable condition for the formation of a "sol" of ferric hydroxide and "humus." This "sol" on reaching the water level accumulates and during the dry months deposits as an irreversible "gel." This latter process also takes place through the action of electrolytes. These authors further say that

as soon as the level exists at all, it is easy to imagine the rapid manner in which it (the level) will increase, and it is not difficult to conceive of the action described going on year after

year until the result called hard-pan is reached. On arrival of the wet season, the coagulated and desiccated colloids will not entirely go back into suspension as the colloidal character may well have been changed during the process of desiccation.

This explanation seems more logical, especially in view of the fact that the authors have been able to obtain these "sols" by washing ferric humus gels.

For the past several months this laboratory has been studying the iron depositing bacteria in relation to their occurrence in milk and dairy products. The writer's attention was first drawn to their general importance through the reading of Harder's (4) paper, in which he gives rather conclusive proof of the possible formation of certain sedimentary iron ores through the agency of the iron depositing bacteria. It was thought that they might as well be a factor in the formation of iron hard-pan which is so prevalent in the west. It is of great interest then to find on reading the paper by Morison and Sothers (7) at the end of their discussion a statement that

it is conceivable that some of the iron bacteria may play a part in the formation of pan, because that will be the only layer which will hold enough moisture for their existence. It is possible that, in the absence of much organic matter, organisms might use the humic acid combined with the iron as a source of energy, and leave the iron in the form of ferric hydroxide. The authors, however, consider that it is possible to account for the formation of pan without the intervention of living organisms.

This statement is made without apparent experimental evidence, and it seems likely that had published evidence existed, the authors would have cited it. To this might be added a recent contribution by Swinerton (8) which, again, is unsupported by experimental evidence but nevertheless shows the trend of thought concerning the iron depositing bacteria. Describing the finding of iron bacteria in Yosemite Valley, he says that

Bacteria, iron bacteria, among many other forms, may be important factors in the cementation of sand and gravel materials. Such would be the logical conclusion to draw from the results of previous studies in bacterial deposition; not only does the activity of the low forms of life provide material for the mass of rocks themselves, but also the material for binding clastic sediments.

Although it is not felt that there is yet direct proof of the contention the author is about to make and, although much more work remains to be done, still it is felt that these observations might add significance to whatever knowledge has been gained concerning the process of the formation of hard-pan.

As long ago as 1836, Ehrenberg suggested that bacteria play an important rôle in the formation of bog iron ore. Molisch (6), Cholodny (2), Ellis (3), and Harder (4) have all worked with these organisms, publishing descriptions of them and of their physiological reactions whenever they were able to propagate them artificially, which is difficult to accomplish. The characteristic reaction seems to be a precipitation of iron salts, usually the hydroxide from solutions of iron. Winogradsky claims (9, 10) that this deposition is a vital process, the organisms receiving their energy from the

reaction, although of recent years the tendency has been to consider that the deposition is simply incidental to the life processes of the organism. Whatever the underlying principle of the precipitation, the fact remains that such a precipitation takes place. The organisms usually defined as the "iron bacteria" are not the true bacteria (*eubacteria*) but probably would be included under the family *Chlamydobacteriaceae* since they are sheathed and filamentous forms. These workers, however, have found that some of the common soil and water bacteria also are able to deposit iron from solution in a manner similar to that of the "true iron" types. The finding of such iron-depositing bacteria in the soils and water of California led to a study of the hard-pans of the state. Several hard-pans were obtained, and from a freshly fractured surface small amounts were ground off with a sterile drill into a medium which has the following composition, as described by Harder (4): K_2HPO_4 0.5 gm., $MgSO_4$ 0.5 gm., NH_4NO_3 0.5 gm., $CaCl_2$ 0.2 gm., $NaNO_3$ 0.5 gm., ferric ammonium citrate 10 gm., water 1000 cc., pH 7.0.

This was used as a broth as well as a solid medium. In figure 1 of plate 1 are shown four of the pans, one of which has two holes, the result of the sampling process. The powdered pan, placed in sterile petri dishes and in tubes of Harder's broth, gave results shown in plate 1, figure 2, and plate 2, figure 1, respectively. The colonies are reddish, irregular, and incrustated with a fine film of iron salts. The broth tubes show the typical deposition of the iron from solution. Incubation to obtain the typical reaction varied somewhat from three weeks to three months.

The numbers varied from a few hundred to many thousand to each gram of pan substance, but since no method of obtaining exact numbers has been evolved, the actual counts are not given.

Isolation of the organisms discloses the fact that rods and cocci both are found in the cultures. They precipitate the iron in pure culture, but at a considerably slower rate. As a class, they ferment the salts of the organic acids with a distinct shift of the hydrogen-ion concentration to the alkaline side. They thus conform to the alkali bacteria, a group of organisms found in milk, and described by Ayers, Rupp, and Clemmer (1). These organisms are being subjected to more careful study which will be reported in a later paper.

In addition to the foregoing, there is further evidence of the possibility of a biological phase in the formation of iron hard-pan when the pans are examined under a microscope. Under a low power dissecting microscope giving magnification of 60 diameters and with reflected light, the pans show a coarse open structure with numerous cavities. These cavities are sometimes seen to be filled with a cobweb-like formation, as is shown in plate 2, figure 2. Only two pans of the six examined showed this formation but it is mentioned as further evidence of the presence of such organisms in pan material. When these filaments are removed from the pan and further examined with the higher power microscope, they appear to be somewhat similar to the higher bacteria.

forms described by Harder and others. Plate 2, figure 3 shows them under a magnification of 900 diameters.

In hard-pan then, we have evidence of both a previous microbial life and a present life, both of which deposit iron from solution. Such facts are of considerable significance. It seems to the author that there is thus offered a plausible explanation of at least one factor in the formation of hard-pan. He has seen in many parts of California, along the roots of grasses, particularly of rice, long tubules of 'red material which are teeming with iron depositing bacteria. Could not the iron which is found in the pan be first precipitated by the organisms and thus carried to the level, which later forms the hard-pan itself? The imagination is not unnecessarily stretched by such a conception.

REFERENCES

- (1) AYERS, S. H., RUPP P., AND JOHNSON, W. T. J. 1919 A study of the alkali-forming bacteria found in milk. U. S. Dept. Agr. Bul. 782.
- (2) CHOLODNY, M. 1926 Die Eisenbakterien. Jena.
- (3) ELLIS, D. 1919 Iron Bacteria. New York.
- (4) HARDER, E. C. 1919 The iron depositing bacteria and their geologic relations. U. S. Geol. Survey Prof. Paper 119.
- (5) HILGARD, E. W. 1914 Soils, p. 183. New York.
- (6) MOLISCH, H. 1910 Die Eisenbakterien. Jena.
- (7) MORISON, C. G. T., AND SOTHERS, O. B. 1914 The solution and precipitation of iron in the formation of Iron Pan. *Jour. Agr. Sci. (England)* 6: 84-96.
- (8) SWINERTON, A. C. 1926 Iron bacteria. *Science*. 63: 74.
- (9) WINOGRADSKY, S. 1888 'Ueber Eisenbakterien. *Bot. Zeit.* 46: 262-270.
- (10) WINOGRADSKY, S. 1922 Eisenbakterien als in Orgoxydanten *Cent. Bakt.* (II) 57: 1-21.

PLATE 1

FIG. 1. Four of the hard-pans used in the studies.

FIG. 2. Typical fermentation of the iron depositing bacteria studied.



FIG. 1

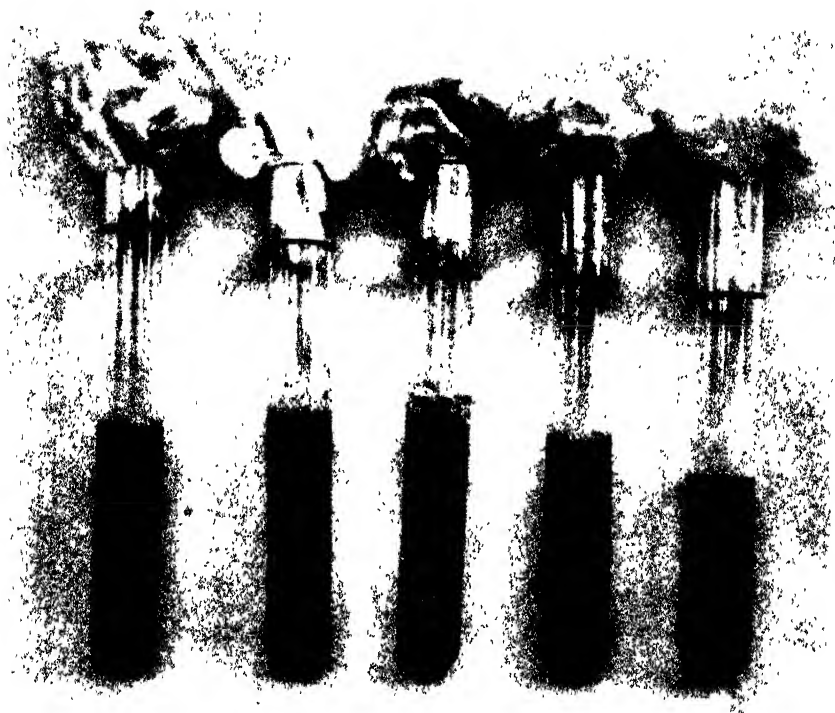


FIG. 2

PLATE 2

FIG. 1. Colonies obtained by plating powdered hard-pan on Harder's medium.

FIG. 2. Filaments within cavities of the hard-pan. Magnification 60 \times .

FIG. 3. Filament seen in figure 2 removed from the cavity and enlarged to 900 \times .

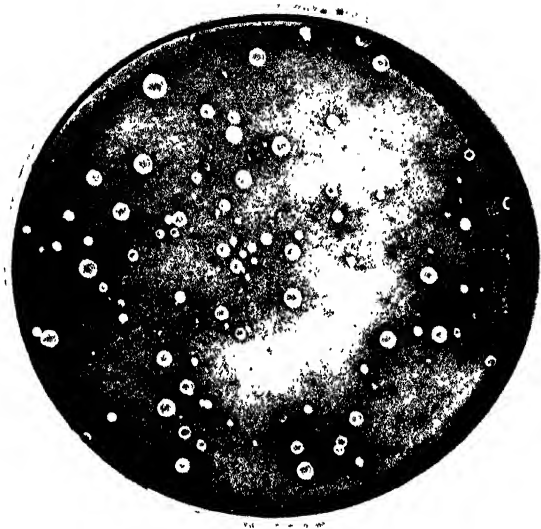


FIG. 1

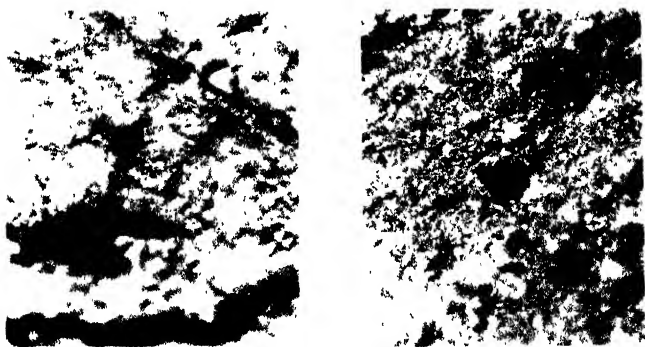


FIG. 2



FIG. 3

A STUDY OF THE SOILS OF HIDALGO COUNTY, TEXAS, AND THE STAGES OF THEIR SOIL LIME ACCUMULATION

HERMAN W. HAWKER

Texas Agricultural Experiment Station

Received for publication August 13, 1926

Hidalgo County, Texas, is located in the southernmost part of the state, about 30 miles west of the Gulf of Mexico, and adjacent to the Rio Grande, which divides it from Mexico. The ninety-eighth meridian of west longitude passes through the eastern part of the county.

The apex of the Rio Grande delta begins near the western edge of the county, the triangle on the American side widening gradually at first, then more rapidly, until at the eastern edge of the county the area of alluvial deposits, including those in both first and second bottom positions, is more than 25 miles wide. The greater part, if not all, of the remainder of the geological material underlying the soils of the county are included in a huge alluvial fan, deposited by drainage waters flowing over the region from the highland northwest of the Rio Grande basin. All of the geological deposits from which the soils of the county have been developed are thus of fluvial origin. The soils on the recently deposited and more or less frequently overflowed alluvial plains are identical with the alluvial deposits, but the rest of the soils of the county have characteristics of great significance, which the alluvial soils do not have. The geological deposits from which the soils in all parts of the county have been developed were, and where not subsequently changed are, calcareous.

The average annual rainfall of Hidalgo County ranges from less than 20 inches in the western part to 22½ inches in the eastern part. Thus the soil material under discussion has existed entirely under semi-arid and semi-humid conditions.

The soils of the county vary from young to mature, the latter being soils which have well developed profiles. The youngest soils of the county, measured in terms of the imperfection of their profile development, are found in the Rio Grande and Harlingen series, which exist as strictly first bottom soils, subject to ordinary overflows by the Rio Grande. Next older are the Laredo soils, existing as natural levees, mounds, and ridges in the first bottom of the river, and subject only to overflow by floods higher than the average. The Hidalgo and Victoria soils occur on terraces or second bottoms, and have had time to develop moderately well defined but incomplete profiles, whereas the Brennan, Willacy, Duval, and Nueces soils have developed profiles that seem to have attained the features of maturity for the environment in which they have developed.

SOIL SERIES

Short descriptions of these soils as found in Hidalgo County follow:

Rio Grande series. Dark brown to brown soils, overlying brown to light brown subsoils. Material of varying colors and textures frequently interstratified. Calcareous from surface downward. Topography flat to nearly level. Drainage only fair to good. First bottom, subject to ordinary overflows.

Harlingen series. Ashy grayish brown to grayish brown soils, underlain by ashy grayish brown to grayish brown subsoils. Soil and subsoil colors brown when moist. Calcareous from surface downward. Topography flat to nearly level. Drainage only fair to poor. First bottom, subject to ordinary overflows.

Laredo series. Brown or light brown to slightly chocolate brown soils, the subsoil being light brown to yellowish brown. Layers of lighter texture than the surface are generally found within three feet of the surface. Calcareous from surface downward. Levee positions or mounds or ridges in first bottom. Subject only to highest overflows of river. Drainage fair to good. Topography nearly level to slightly moundy.

Hidalgo series. Soils brown to slightly dark brown, overlying a friable brown to light brown subsoil which passes through yellowish-brown into a buff or pinkish-buff highly calcareous layer within 3 feet. Soil is always calcareous above 18 inches and generally from surface downward, lime content increasing with depth. Second bottom or terrace position. Nearly level to gently undulating topography. Drainage good as a rule.

Victoria series. Soils dark brown to nearly black, overlying material identical with the lower material of the Hidalgo series. Position, drainage, and topography similar. Soil lime characteristics identical with that of the Hidalgo soils. Eastern correlative of Hidalgo soils.

Brennan series. Grayish brown to brown soils, overlying brown, friable subsoils, becoming light brown or yellowish brown within 3 feet. Not generally calcareous above 30 inches, though sometimes below 18 inches. Below 3 feet a buff-brown or pinkish-buff highly calcareous clay loam is found, which continues to caliche, found at an average depth of 6 feet. Well drained. Gently undulating topography.

Willacy series. Dark brown soils, overlying brown, friable subsoils, becoming light brown or yellowish-brown within 3 feet. Below 3 feet a buff-brown or pinkish-buff highly calcareous clay loam is found, which continues to 10 or 20 feet, when it passes into yellowish-brown to yellow sand. No caliche under these soils. Not generally calcareous above 30 inches, though sometimes below 18 inches. Well drained. Gently undulating topography. Eastern correlative of Brennan soils, except for absence of caliche.

Duval series. Brownish-red to light reddish-brown soils, overlying non-calcareous, friable, light red to red subsoils. Caliche at 3 to 6 feet. No effervescence with hydrochloric acid above caliche. Topography nearly level to gently undulating. Drainage good.

Nueces series. Light brownish-gray to light grayish brown soils, underlain at from 2 to 6 feet by a heavy bluish-gray clay mottled yellow and sometimes red. Underlain at from 4 to 8 feet by caliche. Non-calcareous above caliche. Topography nearly level to undulating, slightly dune-like in places because of reworking of surface material by winds. Drainage good to excessive.

The average annual rainfall of 20 to 22½ inches to which these soils have been subjected since their deposition has been insufficient to remove the soil carbonates present in the soils when they were deposited or those carbonates which have developed through the normal chemical processes continually going on

within the soil mass. These soils, however, vary with age as to the presence and absence of lime in the soil, and the degree to which the leaching and accumulation of the soil carbonates has gone on.

The soils of the county may be divided into two groups on a basis of the presence or absence of carbonates in the upper soil horizons. Using an arbitrary

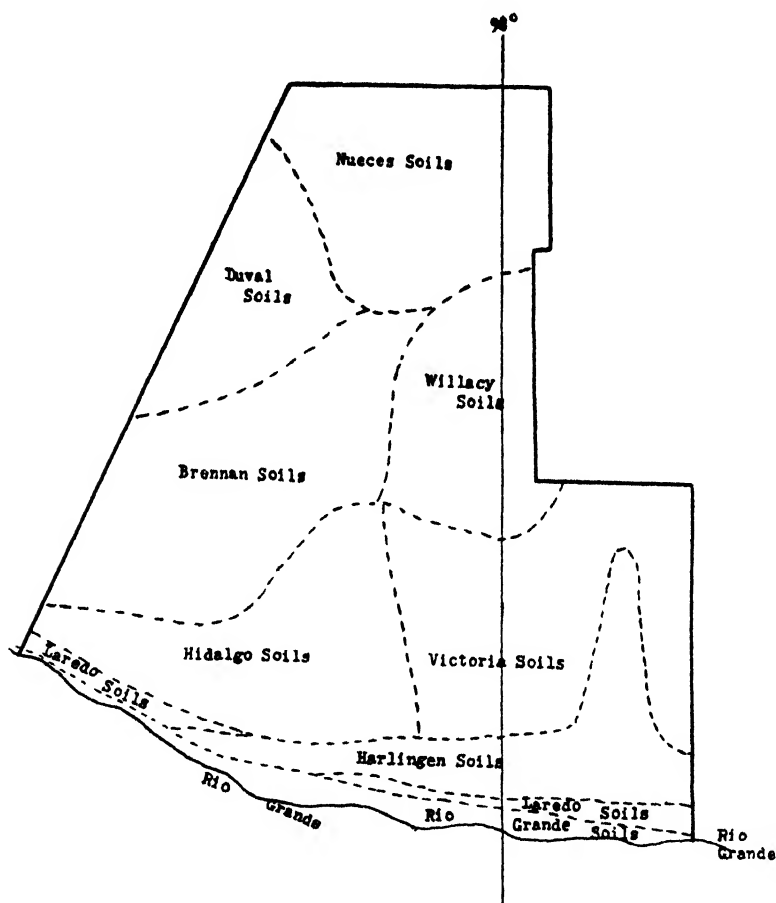


FIG. 1. SKETCH MAP OF HIDALGO COUNTY, TEXAS, SHOWING THE LOCATION OF ITS MAJOR SOIL AREAS

Scale, approximately 8 miles to 1 inch

limit of 18 or 20 inches, those soils having soil lime in the horizons above include the younger soils lying at the lower elevations; namely, the Rio Grande, Harlingen, Laredo, Hidalgo, and Victoria soils. Those soils in which no lime is found above that limit are the older, higher lying soils of the Brennan, Willacy, Duval, and Nueces series.

Even within this broad classification there are distinct differences in the stages of leaching or lime accumulation, which for the most part vary with the age of the soils, the degree of accumulation corresponding in the main with the soil age. A minute examination of an exposed soil profile shows interesting factors, which will be given below.

STAGES OF LEACHING

First stage

In the most recent alluvial soils, those of the Rio Grande series, there is absolutely no evidence of leaching or greater accumulation of soil lime in any particular location or horizon within the section, approximately the same degree of effervescence with hydrochloric acid being obtained throughout. Neither are lime accumulations in the form of concretions or nodules of the smallest size visible. The same is true of the Harlingen soils in all respects, though there is noticed at depths of from five to more than six feet a layer of reddish material. If this reddish material is an evidence of accumulated lime or a distinct high lime bearing horizon it shows a stage of leaching in advance of the Rio Grande series. In this case, however, there is the possibility of this material being an older deposition of reddish material, which would offset the pinkish color found in most cases as incidental to a relatively high stage of lime accumulation in this general region, and indicate that the Harlingen has the same degree of leaching as the Rio Grande. The absence of leaching, or rather the uniform presence of lime throughout the soil profile may be termed the original or first stage in the degree of leaching and lime accumulation.

Second stage

The material of the Laredo series shows a fairly uniform reaction with hydrochloric acid throughout the section. There are visible, however, occasional small lime concretions or accretions, a slight cementation of the soil particles adjacent to old root channels and animal burrows, and a white mycelioid development of soil salts of calcareous nature embracing areas of a foot or more in diameter, generally located more than three feet below the surface. The fact of its greater age as evidenced by its higher position in the Rio Grande first bottom is paralleled by its greater advancement in the leaching of its soil lime and its accumulation in definite areas and forms. This accumulation in the form of minute lime concretions or accretions, a slight cementation of the soil particles in places, and the mycelioid development may be termed the second stage of the development of a definite zone of lime accumulation.

Third stage

A third stage in the degree of leaching and lime accumulation is found in an area of Brennan gravelly soils, found on a terrace in the western part of the county. Gravel and sand strata occur in this phase of the Brennan at a comparatively shallow depth, erosion having been somewhat active at this location. The soil horizon has not been fully leached, since it is still calcareous. A zone of lime accumulation, however, has developed, the carbonates occurring in three distinct forms: first, as a coating around the quartzitic gravel and sand particles, the coating ranging to $\frac{1}{8}$ inch thick in the case of the gravel; second, as a semi-cementing or cementing agency between the sand and gravel, giving the material the appearance of concrete, the term by which this cemented material is known; and, third, in a more or less pure indurated state, or hard caliche, in layers up to six inches in thickness, but of limited lateral extent. The stage of induration reached by the accumulated lime in this location is enhanced by its shallow occurrence, in which position a greater degree of oxidation occurs in a given time, with consequent greater induration. The degree of oxidation here is also increased by the open character of the sand and gravel as it occurred before the accumulation of the leached carbonates began. Under these conditions the degree of accumulation and induration of the carbonates originally present in the soil horizons is not characteristic of the soils of the county.

The characteristic third stage in the degree of leaching of the soil carbonates and their accumulation occurs in the soils of the Victoria and Hidalgo series. Here the soils effervesce with hydrochloric acid from the surface downward, but the strength of effervescence increases with depth, indicating a higher percentage of lime with depth. Small soft lime accretions or concretions are found in the soil mass beginning at from 12 to 18 inches, continuing downward to depths of more than 10 feet, and increasing in size and numbers with depth. At about 12 inches in depth the soil mass in both series becomes brown in color, and with increasing depth the color becomes lighter, passing through light brown and yellowish-brown into a buff or pinkish-buff clay loam at about three feet. This latter material continues without great change to depths of as much as 60 feet according to material taken from wells. Some soft white lime accretions or concretions up to an inch or more in diameter are encountered at lower depths. The buff or pinkish-buff material represents the point of highest carbonate accumulation in soils having reached this stage of leaching and accumulation, and is characteristic of this stage of development of the zone of soil lime accumulation over large areas in southern and western central Texas. Upon exposure to the atmosphere the originally soft white lime accretions or concretions mentioned above become hard on the outside, and, with sufficient time, all the way through. Likewise, the surface of the

buff material becomes slightly hardened and cemented, standing in perpendicular walls for long periods without caving. This hardening on exposure represents a gradation toward the fifth or ultimate, and maximum, degree of leaching of the soil carbonates and their accumulation in definite strata.

Fourth stage

The fourth stage in the degree of leaching of the carbonates from the soil and their accumulation in a definite zone is found in those areas covered by the greater part of the Brennan, and the Willacy soils. Here the soil above 30 inches to 3 feet has been entirely leached of carbonates, no effervescence occurring with hydrochloric acid above these depths. At these depths there is found a yellowish-brown to buff-brown or pinkish-buff layer, identical with that found under the Victoria and Hidalgo soils, which is highly calcareous, and contains hard and soft white lime accretions and concretions varying from $\frac{1}{8}$ to $\frac{3}{4}$ of an inch in diameter. Also there is found a semi-cementation of the material which has filled the smaller old root channels and animal burrows, and whitish seams of highly calcareous material in the finer root channels. In the case of the Brennan soils this highly calcareous material passes at about 6 to 8 feet into white caliche, somewhat indurated in the upper few inches and ranging from 6 to 12 or more feet in thickness. In the eastern correlative of the Brennan soils, the Willacy, this caliche is absent, the buffish calcareous material extending to depths of 18 to 40 feet from the surface, when it generally passes into a yellow calcareous fine sand. The buff material is said by well-diggers to be full of seams of semi-hard, white calcareous concretions or accretions which range up to an inch or more in diameter. It is probable that the higher rainfall under which the Willacy soils exist has had considerable influence on the absence of the caliche layer under this soil, the fine sand occurring beneath the buff layer under the Willacy possibly permitting a wider distribution of the carbonates leached from the soil.

It is distinctly to be understood, however, that the thick caliche bed found under the Brennan and other soils is not regarded as the result of lime leached from the soil alone. This great accumulation must have been aided by subterranean waters of high carbonate content.

Fifth stage

The fifth, or ultimate stage of development of the lime accumulation zone in Hidalgo County is found in the Duval and Nueces soils. In these soils generally no effervescence is obtained with hydrochloric acid above the caliche, and in the exceptions only an inch or two of yellowish or buffish material which is highly calcareous is found above the caliche. This represents the nearest approach to the characteristic highly calcareous buff layer mentioned in connection

with the second, third, and fourth stages. The caliche is found at depths of from 3 to 8 feet under nearly all of the Duval and Nueces soils, being shallower as a rule under the Duval. Especially where it occurs at shallow depths the upper few inches of the caliche is quite hard. Below the hardened caliche will be found a semi-indurated caliche, from 8 to 18 feet thick, in pits, from which it had been removed for road building material. The semi-indurated caliche crumbles fairly readily to particles measuring from one-half to an inch in diameter when newly exposed, but hardens on exposure. Very little change is noted in the character of the caliche throughout the depth of its accumulation.

Where the caliche was penetrated in the northern part of the county there was found a blue, very compact, heavy clay shale, highly calcareous, which seemed to extend to considerable depths.

The face of exposures studied from the level of the indurated lime layer to the surface of the blue clay shale was about 20 feet thick. Aside from the difference in the degree of hardness of the upper part, the upper half or more of this caliche layer presented a practically uniform appearance, and the face exposed had an irregular structure. Beneath this uniform material there were found to be areas of varying extent where there was a columnar structure—alternating columns of caliche extending without change, from the solid mass above down to the level of the blue clay shale, and columns of material of the blue clay shale material extending upward from the shale mass into the mass of caliche. These shale clay columns appeared to be somewhat changed from the underlying mass due to a slight induration and by an accumulation of the whitish lime at the edges of the perpendicular and horizontal cracks which appeared to divide these shale columns into clods approximately an inch in diameter. In general this columnar structure gave the appearance of stalactites of caliche extending from the mass of caliche above into the clay shales beneath, and of stalagmites of the clay shale material extending from the basal material into the mass of caliche. Isolated masses of the clay shale material were found surrounded by caliche, and large masses of caliche were also found entirely surrounded by the clay material.

There is no doubt that the upper part of the caliche mass represents an indurated accumulation of soil lime caused by percolation of soil water downward. The columnar structure seems to indicate either a downward percolation of lime-charged waters through cracks in the clay and deposition of the accumulated lime on the way down, or the rising of subterranean calcareous waters and the accumulation of the material in the columns and the lower part of the caliche mass in this manner.

It will be particularly borne in mind that the five stages of leaching of the soil mass of its carbonates and their accumulation in definite zones as described

above occur within fluvial soils derived from the same or very similar material, under similar conditions of rainfall in a north and south zone less than 40 miles long. The soils are of varying age and elevation, the youngest soils being found at the lowest elevation and the oldest at the highest elevation, respectively. And as has been shown above, the degree of the leaching of the soil of its carbonates with the attendant accumulation in a definite horizon is definitely related to the age of the material.

SUMMARY

Hidalgo County, Texas, is located in the southernmost part of the state, 30 miles west of the Gulf of Mexico, and along the Rio Grande. The ninety-eighth meridian of west longitude passes through the county. The rainfall of the county varies from less than 20 inches in the western part to 22½ in the eastern part, giving a semi-humid to semi-arid climate.

The apex of the delta of the Rio Grande begins in the western part of the county, and widens out to more than 25 miles in the eastern part. All of the geological deposits of the county are of fluvial origin, those of the true delta being alluvial, and most of the remainder lying as a huge alluvial from north-western highlands. The soils on the overflowed alluvial plain are identical with the alluvial deposits, and the remainder have definite characteristics. The geological deposits, where unchanged, are calcareous.

The soils of the county vary from young to mature, depending on the perfection of their profile development. The first bottom Rio Grande and Harlingen soils are youngest; the Laredo occurring in natural levee or high first bottom positions are next; the terrace Victoria and Hidalgo soils with moderately well developed but incomplete profiles are next; and the Brennan, Willacy, Duval, and Nueces soils have attained the mature profile for this section.

The youngest soils have absolutely no evidence of leaching or greater accumulation of soil lime in any particular horizon within the section.

The Laredo soils show a fairly uniform reaction with hydrochloric acid throughout the section. There are, however, small lime concretions and accretions, a slight cementation of soil particles adjacent to old root channels and animal burrows, and a white mycelioid development of calcareous salts at 3 feet below the surface.

The third stage in degree of leaching and accumulation of soil carbonates occurs in the terrace Victoria and Hidalgo soils. These soils effervesce from the surface downward, the effervescence increasing with depth. Small soft lime accretions or concretions are found in the soil mass below 12 to 18 inches, continuing to more than 10 feet, and increasing in size and numbers with depth.

The fourth stage is found in the southern part of the area of Brennan and Willacy soils. Here the soil above 30 to 36 inches has been entirely leached of

carbonates. Below this is found a yellowish-brown to buff-brown or pinkish-buff layer, similar to the lower material of the Victoria and Hidalgo soils, which is highly calcareous and contains much soft white lime in large and small accretions and concretions. Caliche, somewhat indurated in the surface, is found at 6 to 8 feet below the Brennan soils but is absent under the Willacy soils.

The ultimate stage of lime development is found in the Duval and Nueces soils. No effervescence occurs above the caliche, which is found at depths of 3 to 8 feet and is quite hard in the surface at the shallower depths.

These five stages of leaching of the soil mass of its carbonates and their accumulation in definite zones occur within fluvial soils derived from the same or very similar materials, and occurring under similar conditions of rainfall in a north and south zone less than 40 miles in length. The stages are definitely linked with the age of material.

PLATE I

FIG. 1. The third stage of development. The section shown is about 15 feet thick. Soil lime is present from the surface downward, but a definite accumulation begins at about 3 feet, in the light brown to buff material. The small lime accretions or concretions are plainly evident, showing the beginning of soil lime accumulation in a definite stratum, extending from about 4 to 12 feet.

FIG. 2. Caliche bed over 15 feet thick. Where these beds are found the soil section above has been leached of all soil carbonates as a rule. The upper few feet of the caliche has been indurated, whereas that below is fairly chalky, and breaks up into aggregates about an inch in diameter.



FIG. 2

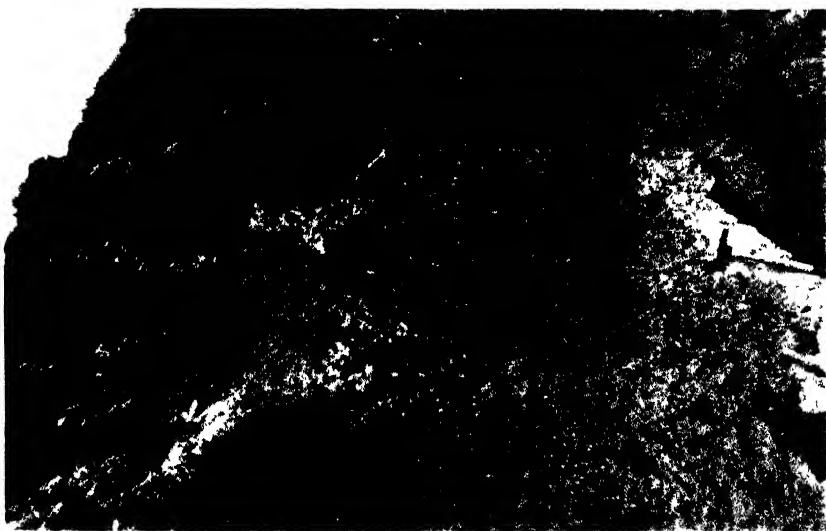


FIG. 1

A STUDY OF THE COLLOIDAL FRACTION OF CERTAIN SOILS HAVING RESTRICTED DRAINAGE

W. L. POWERS

Oregon Agricultural Experiment Station¹

Received for publication August 25, 1926

The movement of water in soils is due to a combination of many factors, among which resistance offered by the amount, composition, and state of colloids present is of chief importance. The other factors commonly listed as affecting percolation are of relatively less importance, or are corollary to the effect of the colloid.

A modern concept of the soil would be to regard it as including a mineral skeleton and a fleshy colloidal covering. If a soil were 99 per cent sand and 1 per cent colloidal clay, the surface area of this finer fraction would be greatest. The colloidal material present is responsible for the shrinking and swelling, the physical absorption, the chemical adsorption, and the base exchange. Any effective applications used to improve soil conditions will accomplish this largely by modifying the state of the colloid present.

When water is applied to a soil, the physical qualities of the soil may be greatly modified. Shrinkage cracks which may affect percolation temporarily or locally are soon swelled shut. Solutes are dissolved and an approximate equilibrium is soon found between the solid phase and the liquid phase, the latter of which is called the soil solution. Removal of solutes like calcium by plant absorption is sufficient to result in a heavier soil structure. If sodium or other monovalent ions invade the system, as when brought in with irrigation water, some increase in deflocculation may be expected. Only a dynamic equilibrium will be found in the soil under crop and the state of the soil colloid will be affected by the kind and concentration of solutes present. There must be some drainage to remove from the system the nonessential deflocculating ion, such as sodium. With the aging of soil, in time a concentration of colloidal material will come about in certain horizons of the soil profile, as best exemplified in mature or aged soil profiles. "Hard pan" layers thus developed may require special treatment. For soil moisture study it would seem best to sample mature soils according to soil horizons and to express results in their ratio to the hygroscopic coefficient.

In a study of percolation of water through soils having a high clay content and restricted drainage, taken from widely separated sections of Oregon,

¹ Released with the approval of the Director, Oregon Experiment Station. Read before American Association of Agriculture Engineers, Lake Tahoe, Cal., June, 1926.

attention has been given to percolation, flocculation, titration, and ion migration or cataphoresis. The soils used included Dayton silty clay loam soil, a heavy alkaline loam from Vale Experiment Field, a colloidal clay from Lower

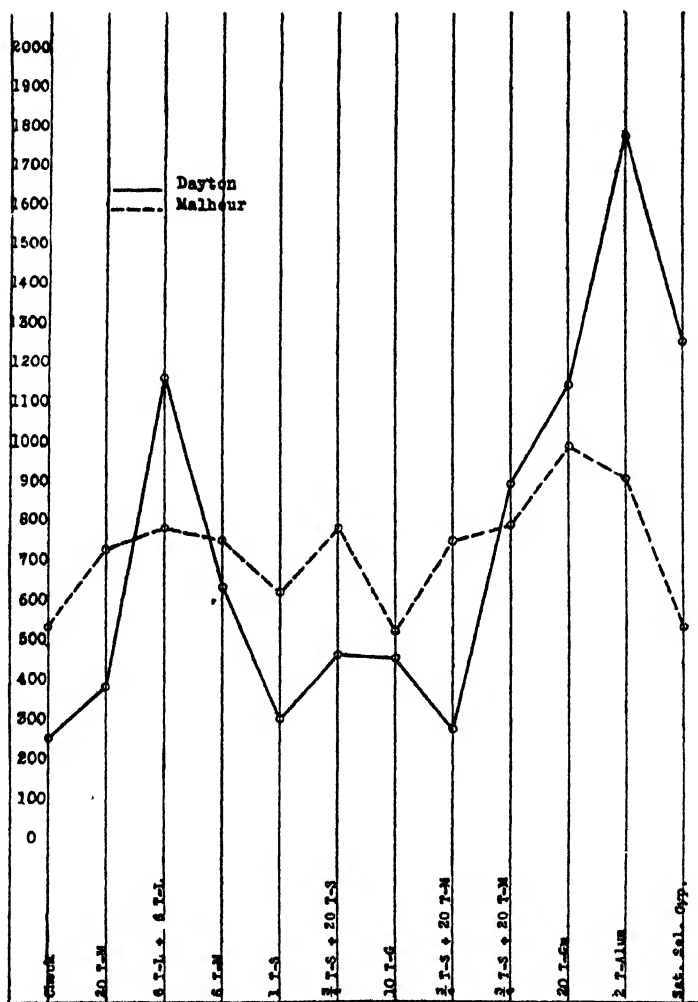


FIG. 1. MAXIMUM PERCOLATION ON DAYTON AND MALHEUR SOILS

Klamath Lake bed, and a gelatinous muck from Lower Klamath Marsh. These soils are described elsewhere (2, 3).²

² The work was done in the Soils Laboratory of the Oregon Experiment Station with the cooperation of two graduate students.

PERCOLATION TRIALS

Twelve 3-gallon jars of Dayton and twelve of the Vale soil were treated as indicated by figure 1, and the maximum rate of percolation was determined. The diagram shows, as found by Young (4),

that all treatments were better than no treatment at all, but that some excelled more than others. The greatest increase in rate of percolation came from the treatments of lime and manure, sulfur and manure, green manure, alum, and a saturated solution of calcium sulfate.

The benefit from the treatment was undoubtedly due to a change in the colloidal state of the clay.

FLOCCULATION

Flocculation studies were qualitative in nature. The solutions used consisted of aluminum chloride, di- and tri-valent sulfate, ferric phosphate, ferric chloride, calcium sulfate, magnesium chloride, lime-water, alum, ammonium nitrate, and three acids—sulfuric, nitric, and hydrochloric. Ammonium hydroxide was used as a dispersing or stabilizing agent. The nearly pure colloid was prepared by churning the soil with several parts of distilled water in a 5-liter bottle, and after some days sedimentation, centrifuging the supernatant suspension through a supercentrifuge. The colloid was collected on a Pasteur-Chamberlain filter candle and thoroughly washed. It was then of the consistency of axle grease. In the flocculation study, 10-cc. portions of the stabilized suspensions of these colloids were used.

It was found that the rate of flocculation of colloids corresponded very closely to the valence of the ion used and the size of the coagulated particles had filled out very directly as the valence of the ion used. When monovalent cations were used a very finely divided precipitate slowly settled out. Lime-water caused flocculation almost as rapidly as alum or other trivalent ions. Flocculation by acids and calcium was more rapid when the solution had been previously made alkaline with ammonium hydroxide. Both colloids showed a close relationship of flocculation to the valency and atomic weight. The beneficial effect from the use of sulfur on the physical condition of the soil of Vale Experiment Field is believed to be due to oxidation of sulfur to sulfuric acid, and the resultant neutralization and increase in calcium dissolved by this acid and brought into solution, the calcium probably being the direct flocculating agent rather than the sulfate ion.

TITRATION STUDIES

Results of titration studies of colloids from these four soils are shown in figure 2, and all show similarity. Differences are attributable to difference in composition and concentration of alkali or acid. At the high acidity it appears that tri-valent iron and aluminum still remain unreplaced from the

colloidal complex. Flocculation may be considered as a neutralization and a cation may be expected to accomplish this for a negatively charged colloid. Hydrogen ion is nearly as effective as bivalent cations for flocculation.

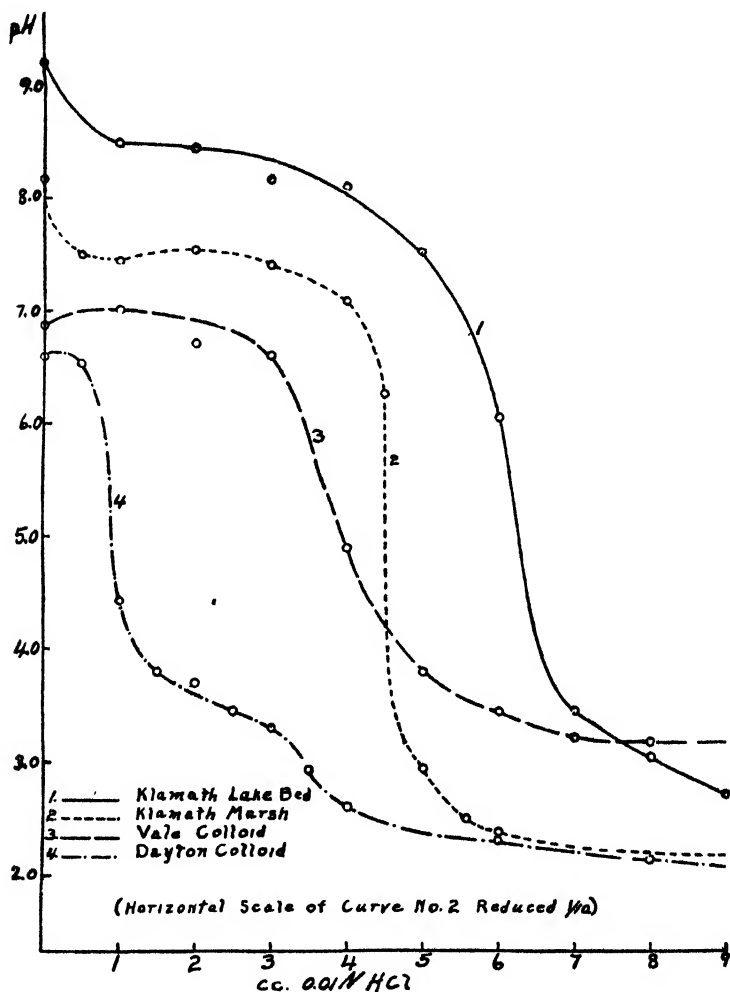


FIG. 2. TITRATION CURVES OF SOIL COLLOIDS

MIGRATION STUDIES

Migration or cataphoresis trials were made for these colloids. At reactions from pH 10.0 to pH 2.5 all these colloids were found to migrate in an electric field as though having a negative charge. It was thought possible that the organic colloid from Klamath Marsh may manifest an isoelectric point, but no indication of this was found.

LYSIMETER STUDIES

Lysimeter studies have been maintained at the Oregon Experiment Station for several years (3). By working with soils of different textures, both with and without crop, it has been found that percolation loss is diminished or eliminated with increasing very fine texture under moderate irrigation and that the crop greatly reduces percolation. At Corvallis, lysimeters filled with two heavy soils; namely, Dayton and Willamette silty clay loams, and then treated some with lime, others with manure, others with lime and manure, and still others untreated, are found by Higby (1) of this department to show that "in all cases lime and manure applications singly or in combination increased the flow of drainage."

FIELD TRIALS

In alkali land reclamation investigations at Vale, Oregon (2) about one ton sulfur or four to five tons of gypsum used with a light application of manure have been effective in improving the structure of sodium saturated "black alkali" land and have made possible the growing of good crops of rye and sweet clover. Alum has been less effective in the field than in laboratory studies. Copious irrigation and pasture growth in natural vegetation are giving promising economic results.

REFERENCES

hⁱ

- (1). HIGBY, W. M. Removal of plant nutrients in percolate from lysimeter tanks. Ore. Agr. Exp. Sta. Bul. in press.
- (2). JOHNSTON, W. W., AND POWERS, W. L. 1924 A Progress Report of Alkali land Reclamation Investigations in Eastern Oregon. Ore. Agr. Exp. Sta. Bul. 210.
- (3). POWERS, W. L., AND CRETCHER, W. 1921 Farm Drainage. Ore. Agr. Exp. Sta. Bul. 178.
- (4). YOUNG, V. D. Improvement in Percolation of Water through Soils Having a High Clay Content and Restricted Drainage. Ore. Agr. Exp. Sta. Unpublished.

BOOK REVIEWS

Grundzüge der praktischen Bodenkunde. By DR. HERMANN STREMMER, Gebäuder Borntraeger, Berlin, 1926. 332 pp., 6 figs., 10 diagr. Price, unbound, R. M. 16.50; bound, R. M. 19.00.

The author of this book, the well known German pedologist, states in the preface that most of the textbooks of soil science devote too much attention to the soil profile and not enough to the surface of the soil, including soil mapping, and that they also lack the practical application of the subject. The book under consideration has been written to discuss largely these points, based upon the extensive experience of the author in mapping soil at Danzig, on the basis of climatic soil types; it embraces also a field study of soils in general. Laboratory methods of analysis are not described.

The author reviews the various soil types, the formation of soils under the influence of climate and ground waters, and then discusses in detail the preparation of soil maps, emphasizing throughout, the application of the results of soil mapping to practical agriculture.

A rather abortive attempt is made to discuss the soil organisms, which are soon dismissed by an indication that there is no single soil constituent which is not acted upon in one way or another by the soil bacteria and other organisms; the author then proceeds to discuss the ecology of higher plants. An equally inadequate attempt is made to discuss the nature of soil "humus," which is referred to merely as a mass of waste products of animal life. Among the soil animals, the earthworms are supposed to have been studied most, their rôle being of course that of "humus formers"—a theory current fifty to sixty years ago. It is also peculiar that a book on practical soil science should not consider the biochemical processes in the soil, although the author indicates that the distinct difference between soils and rocks is due largely to the presence of living organisms in the former.

It is about time that writers of textbooks which are devoted to the subject of soil science in general should consider the activities of the lowly organisms, whose rôle in soil processes is far reaching.

A more appropriate title for this book, which is invaluable to people interested in soil mapping, would be "Characteristics of Soil Mapping and its Application to Practical Agriculture."

SELMAN A. WAKSMAN.

Principles of soil microbiology. By SELMAN A. WAKSMAN, Associate Professor of Soil Microbiology, Rutgers University, and Microbiologist of the New Jersey Agricultural Experiment Station. Williams and Wilkins Company, Baltimore, 1927. xxviii, pp. 897, pl. 19, figs. 82.

Biologists in general and soil scientists in particular should be happy to learn of the publication of this brilliant presentation of the development and present status of the knowledge of the occurrence of microorganisms in the soil, their activities, and their rôle in soil processes.

The volume is divided into 32 chapters arranged under three main sections: (1) occurrence and differentiation of microorganisms in the soil, (2) isolation, identification, and cultivation of soil microorganisms, (3) chemical activities of microorganisms. Quite logically the activities of the microorganisms receive the most extensive and interesting treatment. Among the subjects treated the following may be of particular interest: the soil population—distribution, abundance, and methods of study; autotrophic bacteria; bacteria concerned in the numerous transformations of nitrogen; decomposition of organic materials with particular emphasis upon the influence of nitrogen upon the process; energy transformations in microbial metabolism; transformation of inorganic soil constituents; soil as a habitat for microorganisms causing plant and animal diseases; and soil inoculation. The volume is concluded very originally with a short review of the historical development of the subject. The subject material is replete with references to the literature and well illustrated by plates, line drawings, and graphs. The text is fortunately preceded by a list of some 200 reference books on sciences directly or indirectly related to soil microbiology. The reader has ready access to the text from indices of both authors and subjects.

The author has brought together the information on microorganisms and soil processes in such a manner that one can hardly fail to become impressed with the idea that soil microbiology is a well defined and important science. It is arranged not as a compilation but as a complete and well balanced critical presentation of the facts and ideas concerning the soil population. The method of treatment gives one a new insight into the interpretation of microorganisms as agents in the chemical processes in the soil environment.

Few of the profusion of books recently from the press seem destined to satisfy such a very evident need of long standing for a concise and readily available source of information on the subject. It should be received enthusiastically not alone by soil biologists but all soil scientists as well as individuals interested in biology from the point of view of the distribution and activities of the infinitely small organisms in their relations to soils and plant growth. It is hardly a text book for undergraduate students but rather a general reference text for teachers and research workers.

An unprecedented venture of this kind may justly be expected to be imperfect, but the author appears to have happily developed the subject material with the minimum distortion. The physical makeup is admirable. The

Soils—

dispersion of, 379-388, 391
factors affecting the H-ion concentration of, 399-414

heat of reaction of, 244

heat of wetting of, 244

The Hydrometer as a New and Rapid Method for Determining the Colloidal Content of, (paper) George John Bouyoucos, 319-331

The Hydrometer as a New Method for the Mechanical Analysis of, (paper) George John Bouyoucos, 343-353

The Normal Moisture Capacity of, (paper) Charles F. Shaw, 303-317

The Phenomena of Contraction and Expansion of Soils When Wetted with Water, (paper) George John Bouyoucos, 119-126

Soybean plant, influence of boron on the growth of, 83-105

Specific resistance of dialysate from soil extract, 20-22

Spencer, M. L. (Review of his book) *Données Numériques de Cristallographie et de Minéralogie*, etc., 333

Starkey, R. L., and Henrici, H. T., (paper) The Occurrence of Yeasts in Soils, 33-45

Stremme, Herman (a review of his book) *Grundzüge der praktischen Bodenkunde*, 498

Subsoil—

effect of surface layer treatment on reaction of, 409-412

Studies on the Removal of Nutrients from, by Alfalfa, (paper) C. E. Millar, 261-269

Salts, outgo of, from soils limed with Ca and Mg limes, 187-190

Sulfur and treatment of cotton seed, 1-3, 8

Storage, packing house, for nitrification experiments, 140-160

Temperature—

effect of, on *R. leguminosarum* in, 355-362

effect of soil on, in local areas, 366-376

Thornton, H. G., and Fisher, R. A., (paper) On the Existence of Daily Changes in the Bacterial Numbers in American Soil, 253-259

Urea—

effect of, on seed germination, 337-340
nitrification of, 140-160

Vandecaveye, S. C., (paper) Effect of Moisture, Temperature and Other Climatic Conditions on *R. leguminosarum* in the Soil, 355-362

Waksman, Selman A., (a review of his book) *Principles of Soil Microbiology*, 493-494

Waksman, Selman A., (a review of a section in Abderhalden's *Handbuch der biologischen Arbeitsmethoden*) *Methoden der Mikrobiologischen Bodenforschung*, 495

Waksman, Selman A., and Davison, Wilburt C., (Review of their book) *Enzymes*, 81-82

Water, absorption of, by the lower root system, 266

Water extracts of soils, review of methods, 13-14

Water of adhesion in soil, definition of, 315-316

Weidemann, A. G., See McCool, M. M., and Schlubatis, G., 391-398

Wiegner, George, (paper) Method of Preparation of Soil Suspensions and Degree of Dispersion as Measured by the Wiegner-Gessner Apparatus, 377-390

Wiegner, G., and H. Jenny (Review of their book), *Anleitung zum quantitativen agrikulturchemischen Praktikum*, 415

Wisconsin drift

density of forest and prairie soil on the late, 73-82

the forest floor of the late, 57

Yeasts, occurrence of, in soil, 33-45

INDEX

Sodium nitrate, effect of, on potassium availability, 208-210

Sodium sulfate—

effect of—

- on ammonification, 280-284
- on bacterial numbers, 275-280
- on leaching alkali soil, 274
- on nitrification, 284-287
- on nitrogen fixation, 287-295

Soil—

air, the CO₂ concentration of, 421-424
contraction values of a mineral, with organic matter, 123

CO₂ at different depths in the, 424

density of forest and prairie, 73-82

density of surface, study of, 73

determination of ferrous iron in, 169

Differences in the Heat of Reaction between Artificial and, Gels of Silica, Alumina and Iron with Hydroxides, (paper) George John Bouyoucos, 243-250

effect of air-drying a, on its reaction, 399-401

effect of electrolytes on kaolin and the relation to the, 225-242

effect of grinding a, on its reaction, 401-402

Effect of Moisture, Temperature and Other Climatic Conditions on R. Leguminosarum in the, (paper) S. C. Vandecaveye, 355-362

effect of, on climate, 363-376

evolution of carbon dioxide in, 417-453

fertility of a, as Related to the Forms of Its Iron and Manganese, (paper) P. H. Brewer and R. H. Carr, 165-173

fertility of, and colloidal behavior of soils, 127-135

heating effects on oven-dry, 123

influence of freezing on dispersion of, particles, 382-384

methods of sampling and analyses of forest floor, 60-61

Minnesota Glacial, Studies: II. The Forest Floor on the Late Wisconsin Drift, (paper) Frederick J. Alway and Paul M. Harmer, 57-71

Minnesota Glacial, Studies: III. Density of the Surface Foot in Forest and Prairie on the Late Wisconsin Drift, (paper) Paul M. Harmer, 73-82

moisture, review of literature on, 303-304

Nutrient Deficiency in the Soil—
Method of Determining, 127-135

Ames and R. H. Carr, 127-135

On the Existence of, 127-135

Bacterial, 275-280

H. G. Thomsen and J. A. Harner, 259

Potassium, 280-284

cator, 280-284

J. W. American, 280-284

Studies on, 280-284

Bacteria, 275-280

Feirer, 47-53

The Microflora, 275-280

Leached and, 275-280

(paper) J. E. G. 275-280

The Occurrence of, 275-280

L. Starkey and A. 275-280

the, respiration, 417-453

The Use of Collodion, 275-280

Clear, Extracts for, 275-280

of the Water-Sol, 275-280

(paper) W. H. Pierr, 275-280

13-32

Soil density, method of, 127-135

Soil fertility and respiration, 417-453

Soil gases, method of determining, 417-453

Soil moisture, the influence of, 141-148

tion, 141-148

Soil Profiles, Further Studies, 141-148

with Special Reference to, 141-148

Materials, (paper) M. 141-148

G. Weidemann and G. 141-148

398

Soil Suspensions, Method of, 141-148

and Degree of Dispersion, 141-148

by the Wiegner-Gessner, 141-148

(paper) Georg Wiegner, 141-148

398

Soil toxicity, relation of iron, 141-148

nese to, 165

Soil Zones, Outgo of Calcium, 141-148

Nitrates and Sulfates from, 141-148

Calcic and High-Magnesian, 141-148

incorporated in Two, (paper) 141-148

MacIntire, 175-197

Soils—

A Study of the Colloidal, 141-148

Certain, Having Restricted, 141-148

(paper) W. L. Powers, 141-148

Colloidal Behavior of, and Soil, 141-148

III. Cation Replacement and, 141-148

of Soil with Ca, (paper) 141-148

and H. C. 141-148

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before

Return Date	Return Date